Figure 1: Examples of Nuclease Stable Ribozyme Motifs

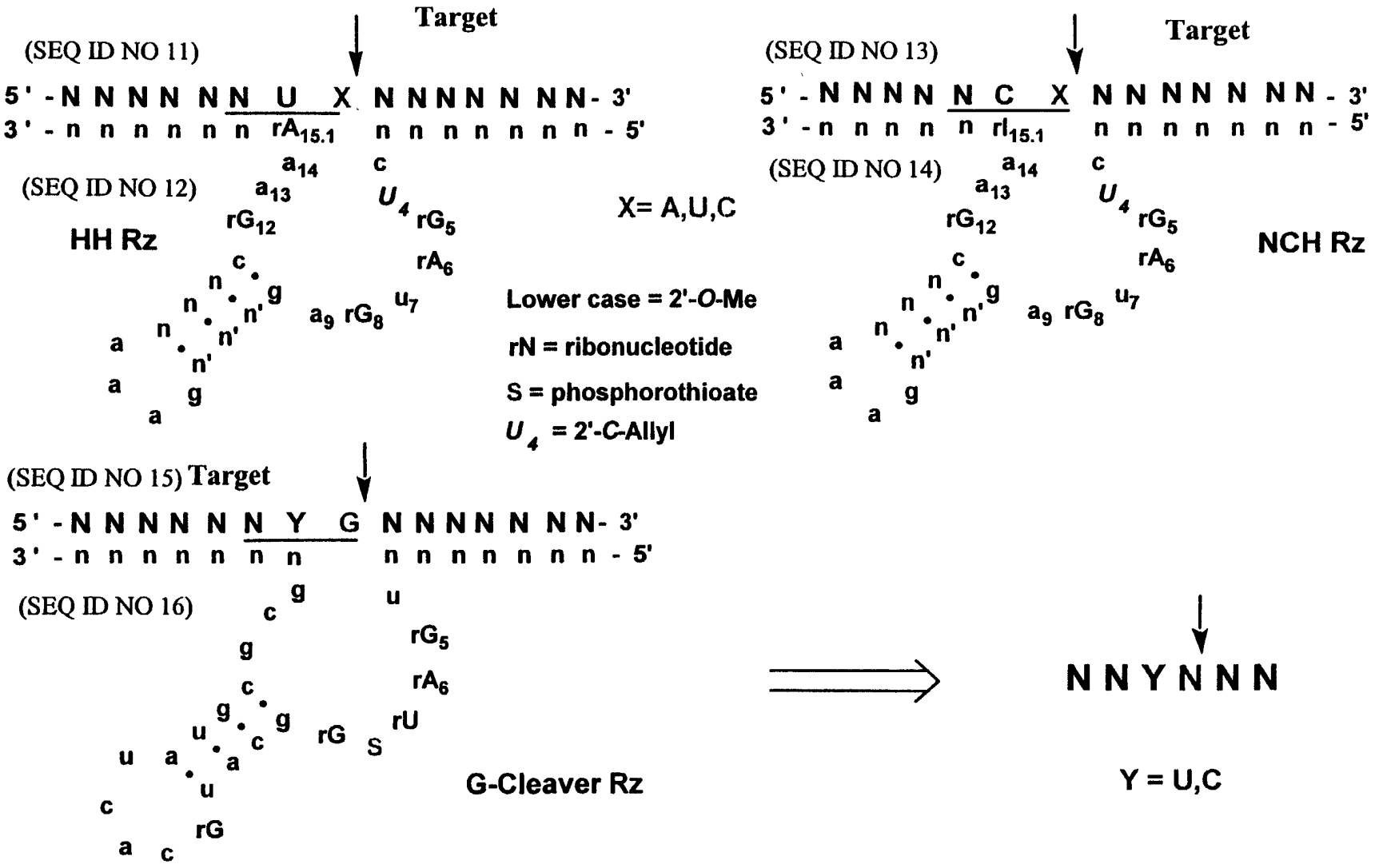


Figure 2: 2'-*O*-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

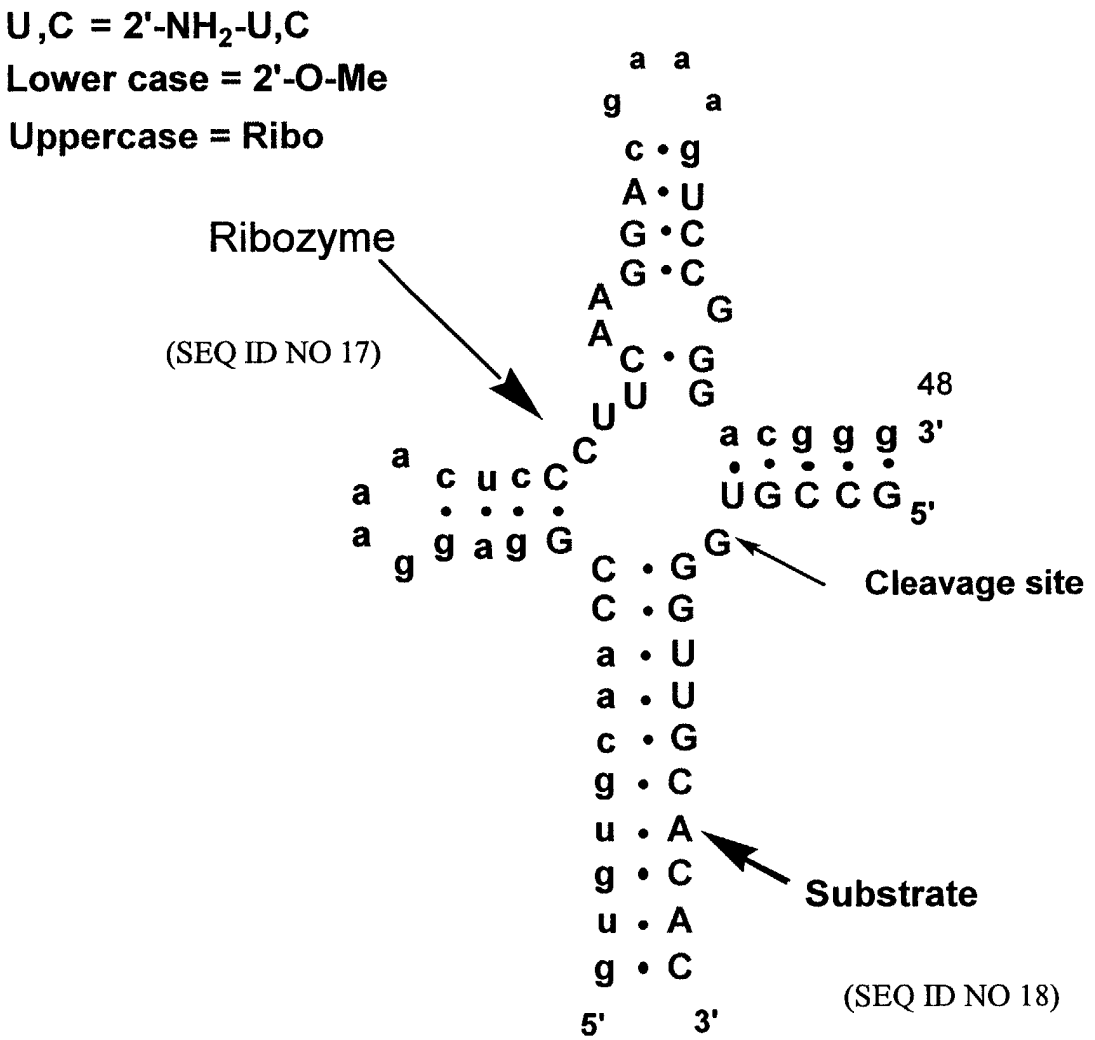
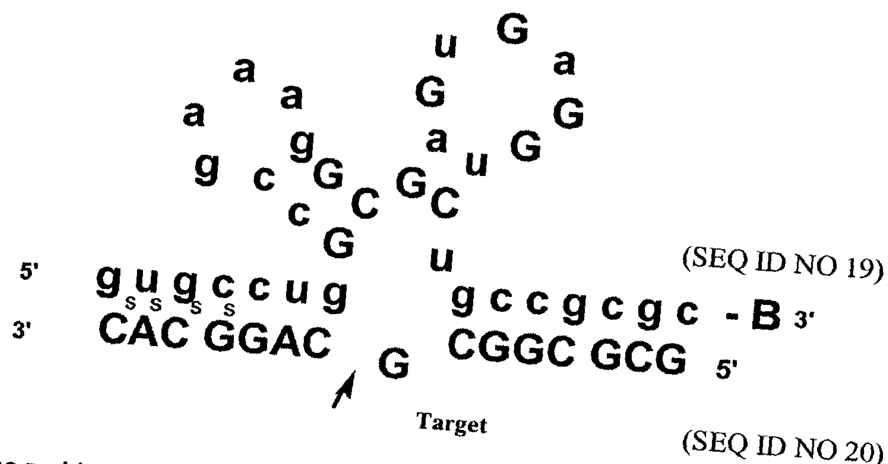


Figure 3: Stabilized Zinzyme Ribozyme Motif

Zinzyme A-motif RZ



Legend

Uppercase indicates natural ribo residues

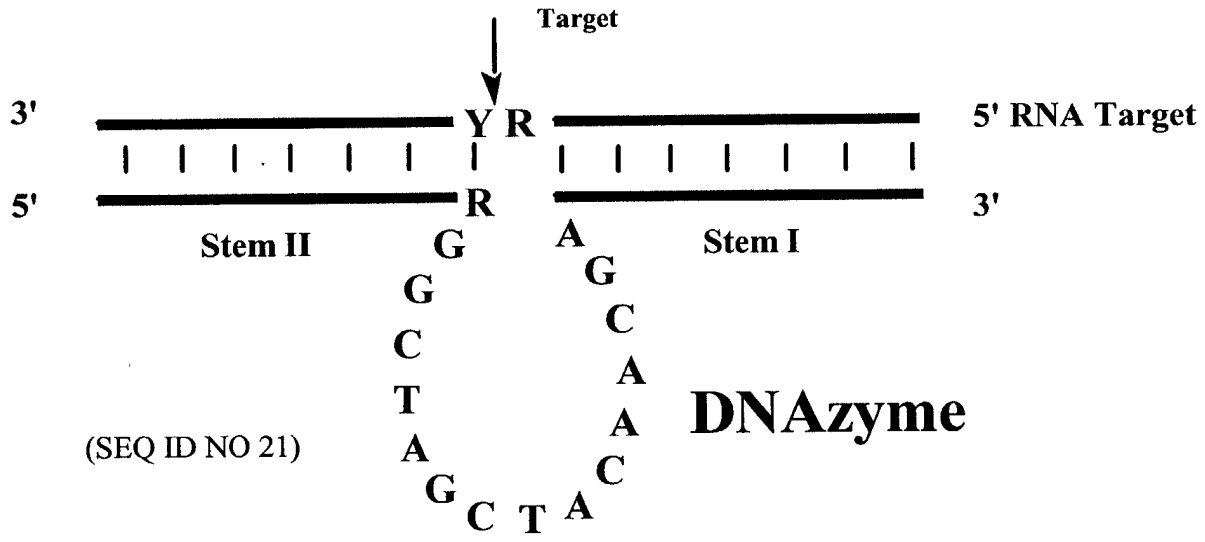
C indicates 2' - d-NH_2-C

Lowercase: 2'-*O*- Me

Subscript $_s$ indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif



(SEQ ID NO 21)

Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

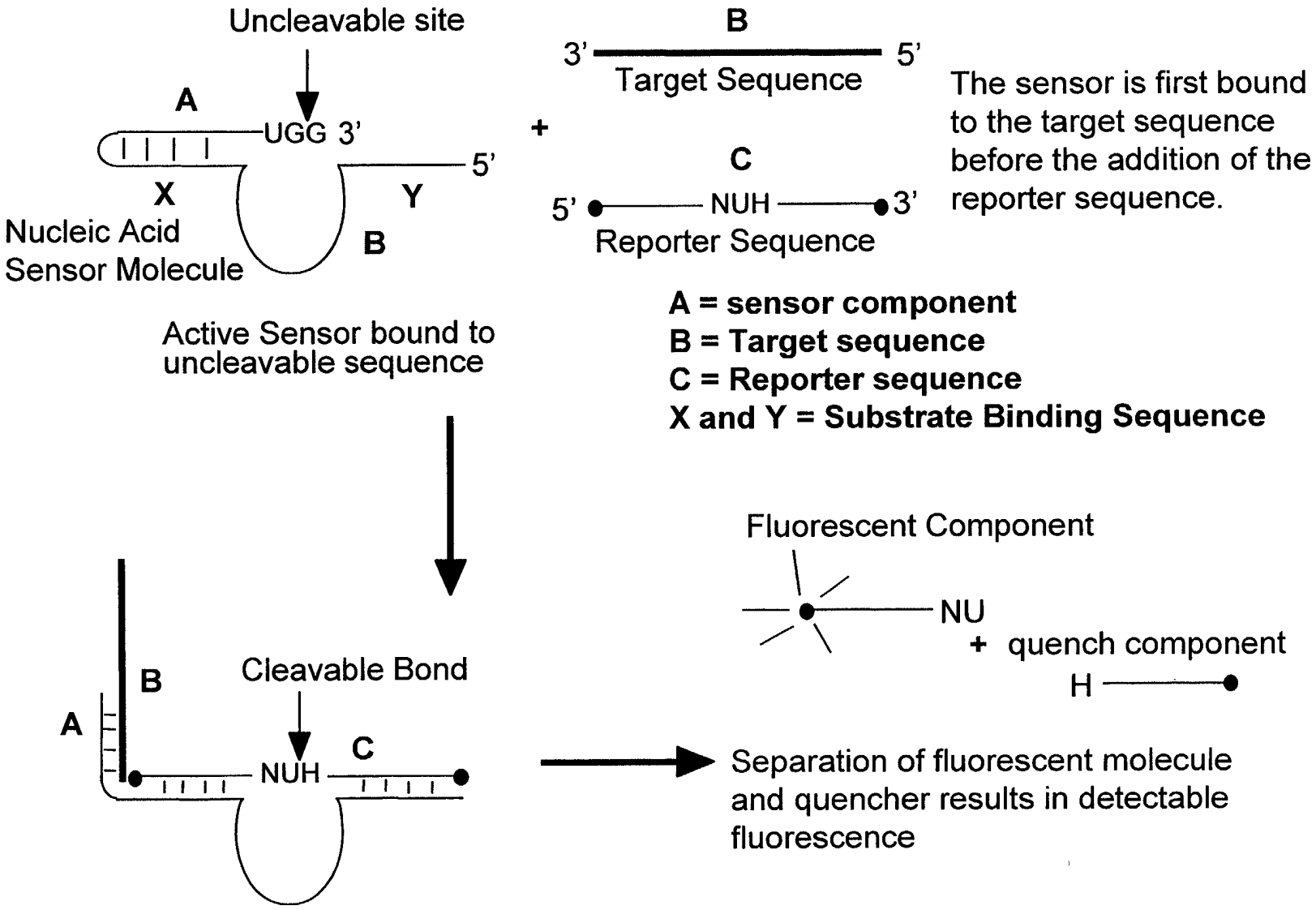


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

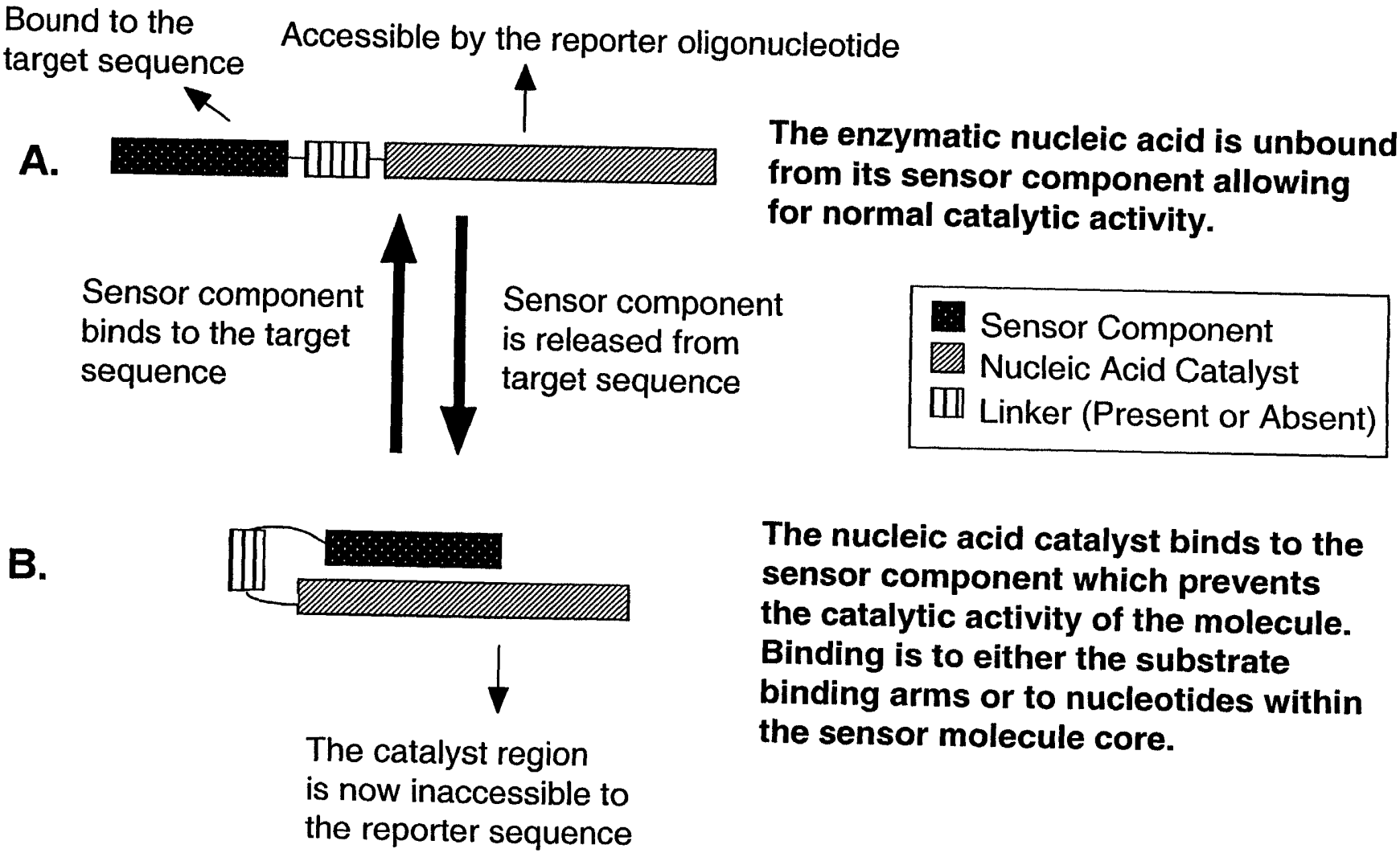


Figure 7a. Examples of Diagnostic Effector Molecules

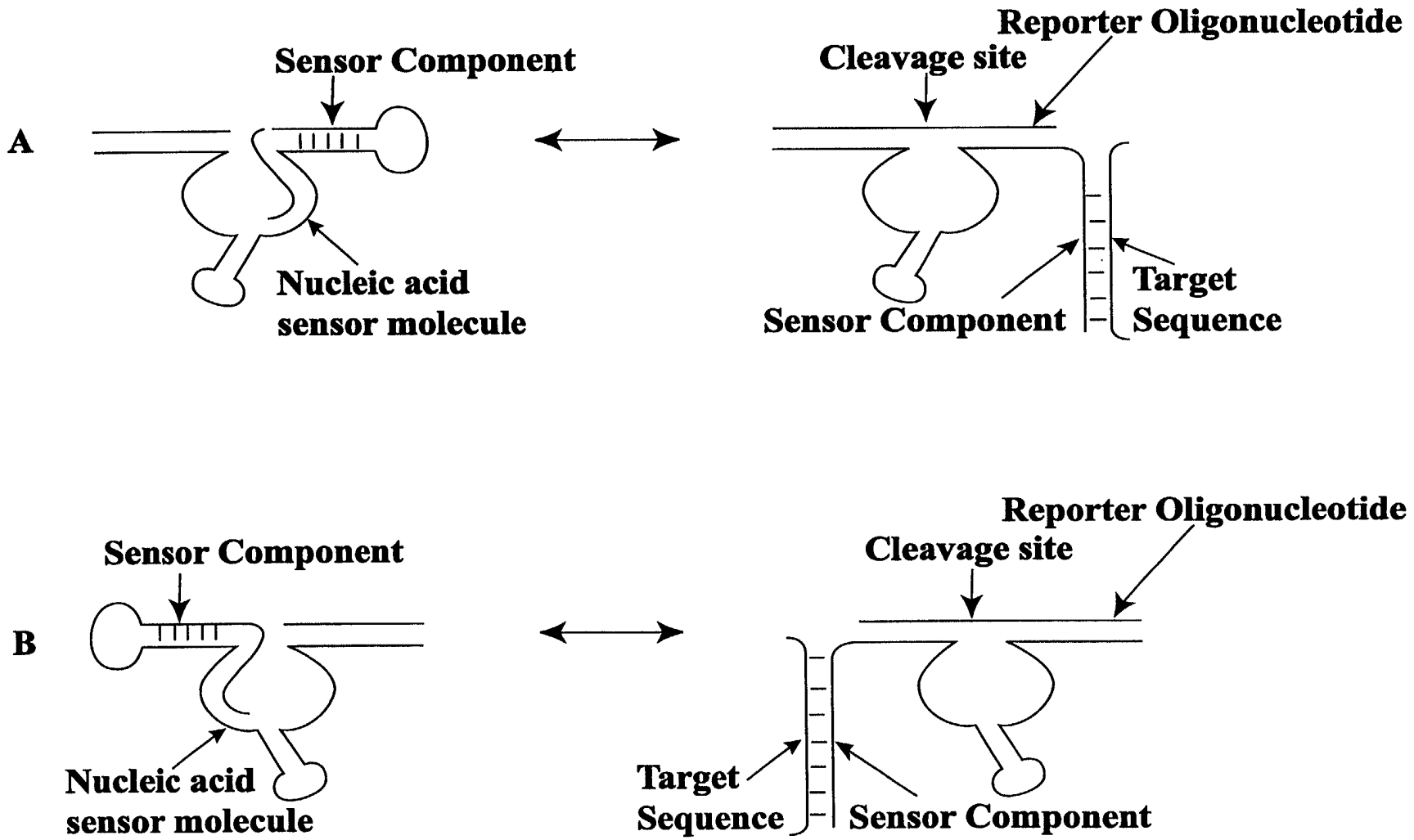


Figure 7b. Examples of Diagnostic Effector Molecules

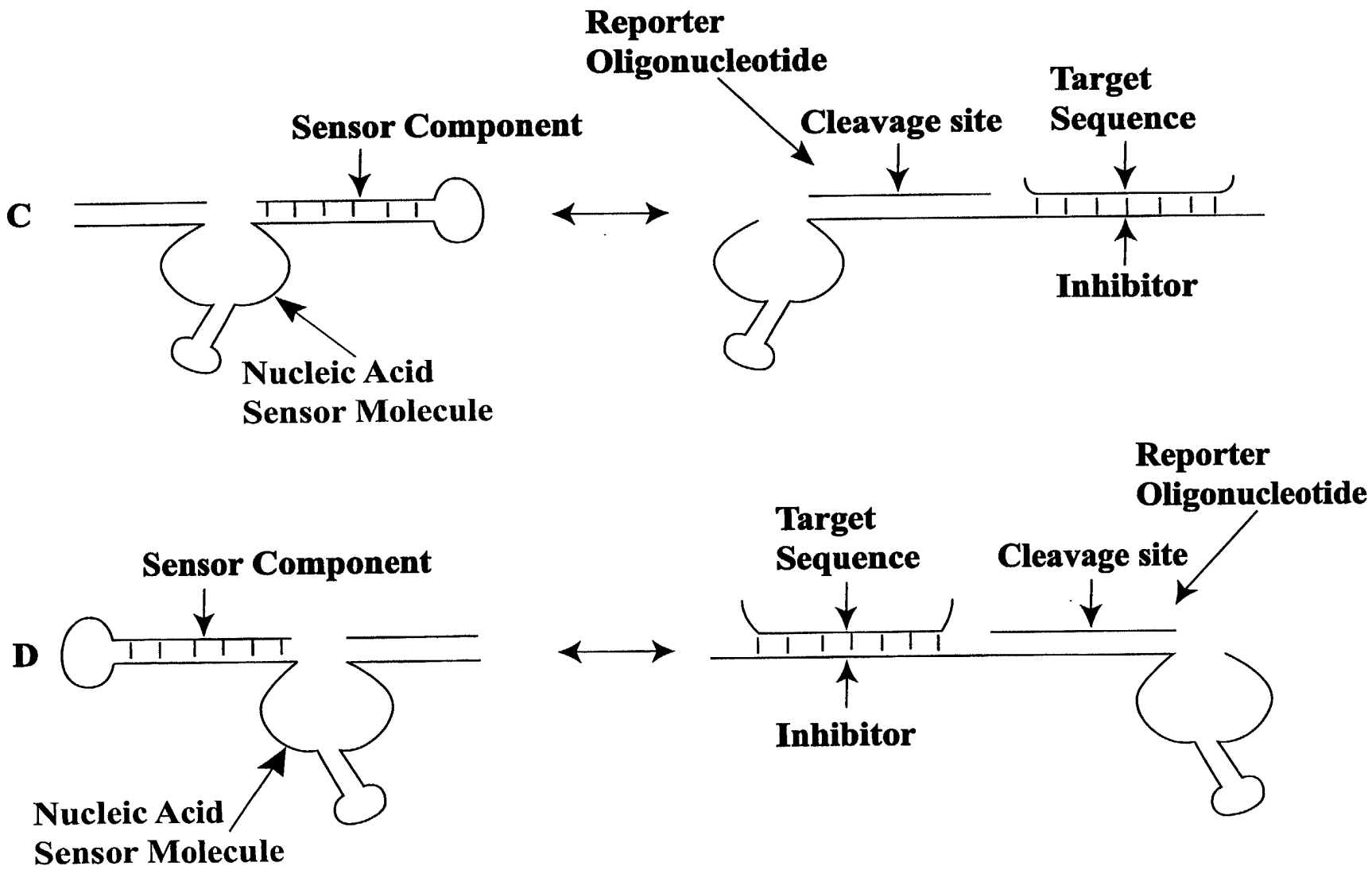


Figure 8a. Examples of Diagnostic Effector Molecules

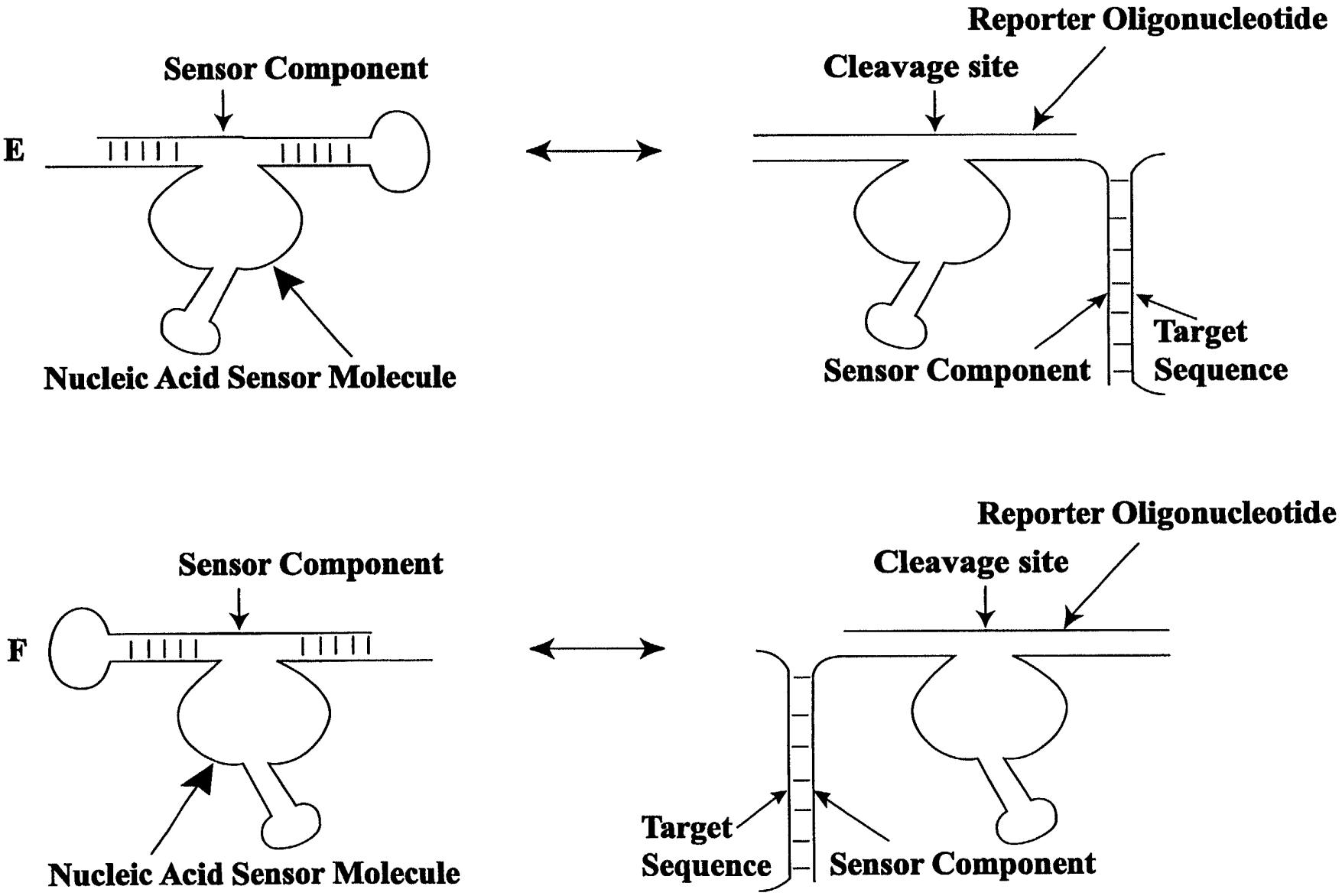


Figure 8b. Examples of Diagnostic Effector Molecules

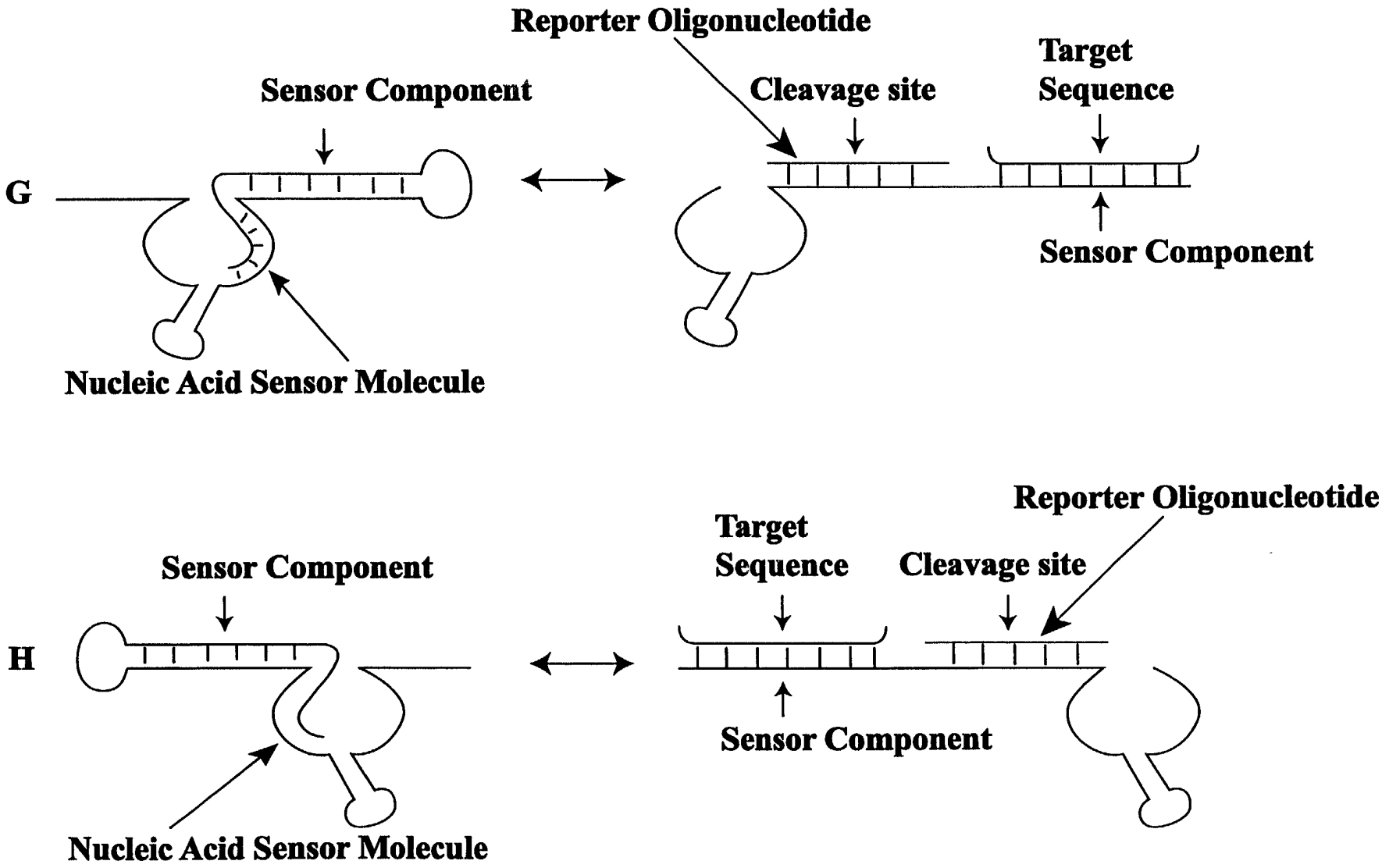


Figure 9. Examples of Diagnostic Effector Molecules

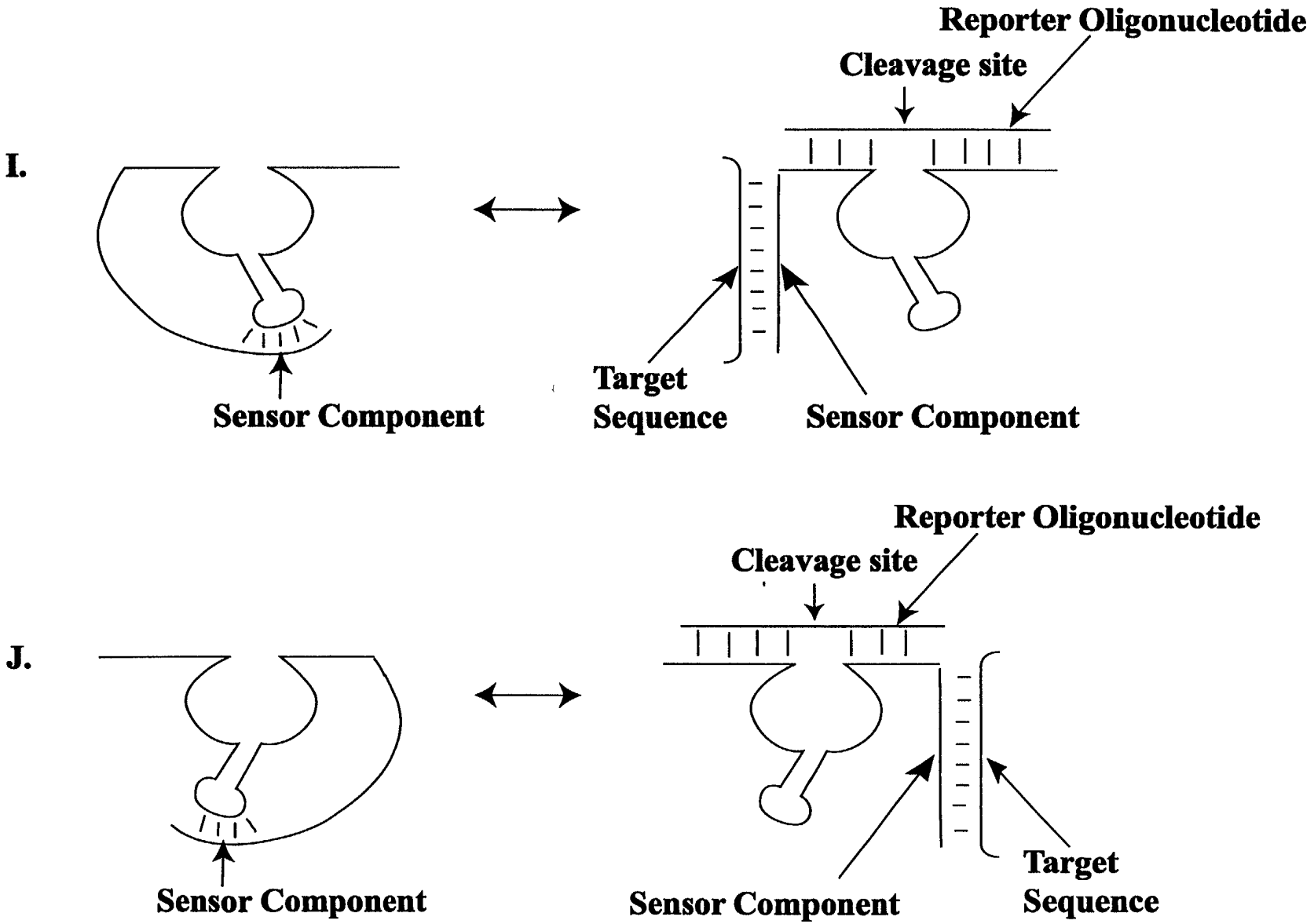
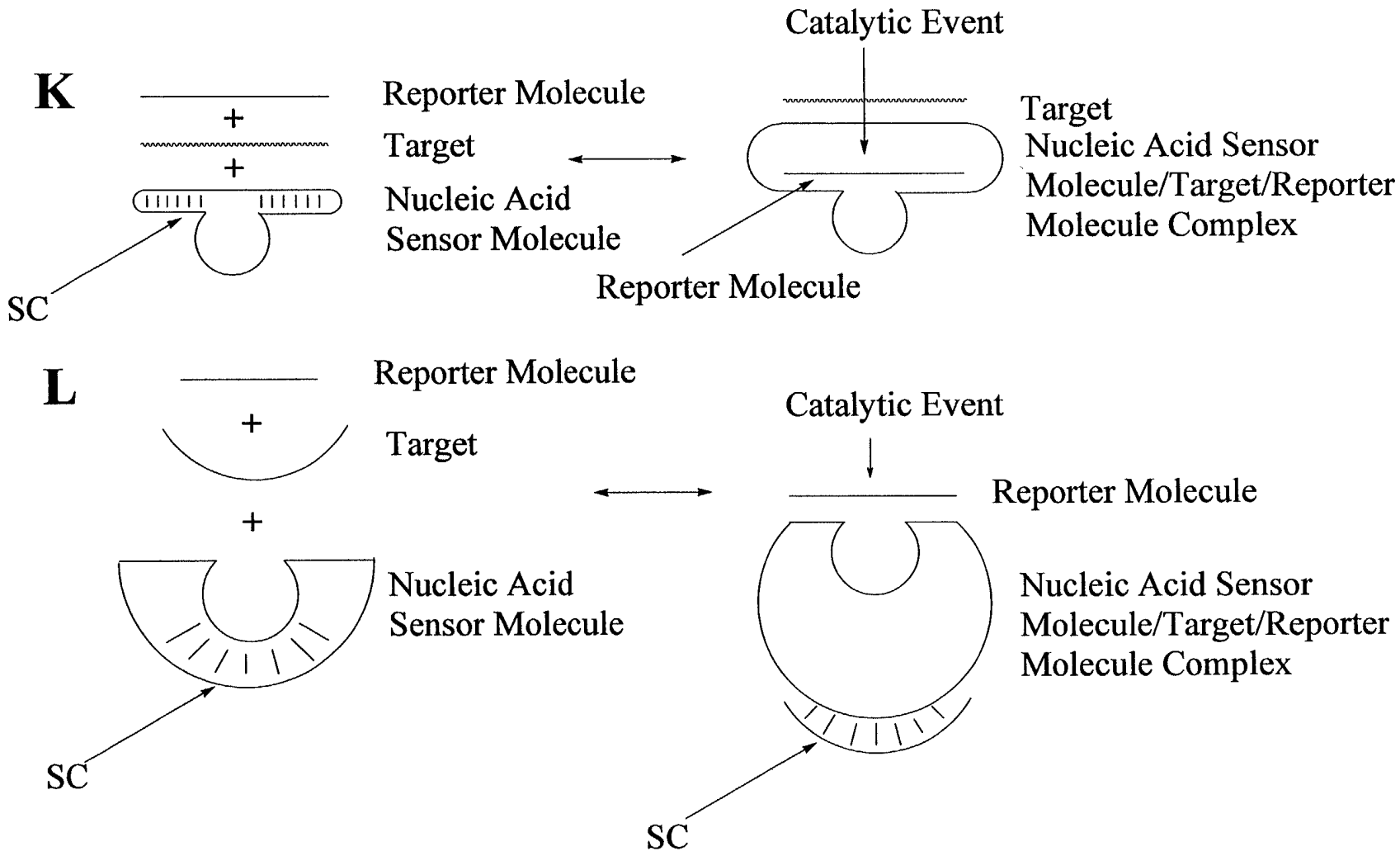


Figure 10: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 11: Examples of Diagnostic Effector Molecules

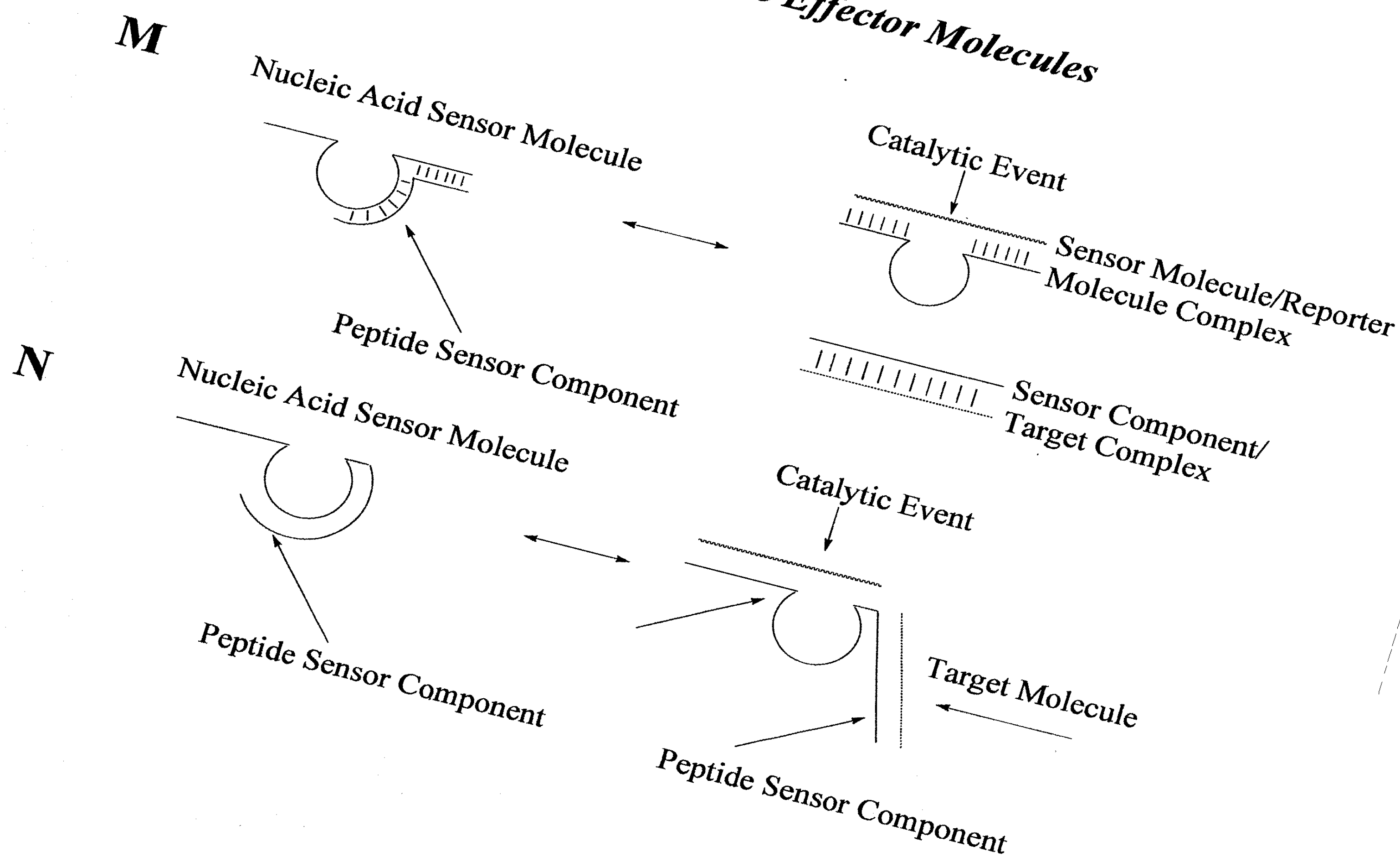
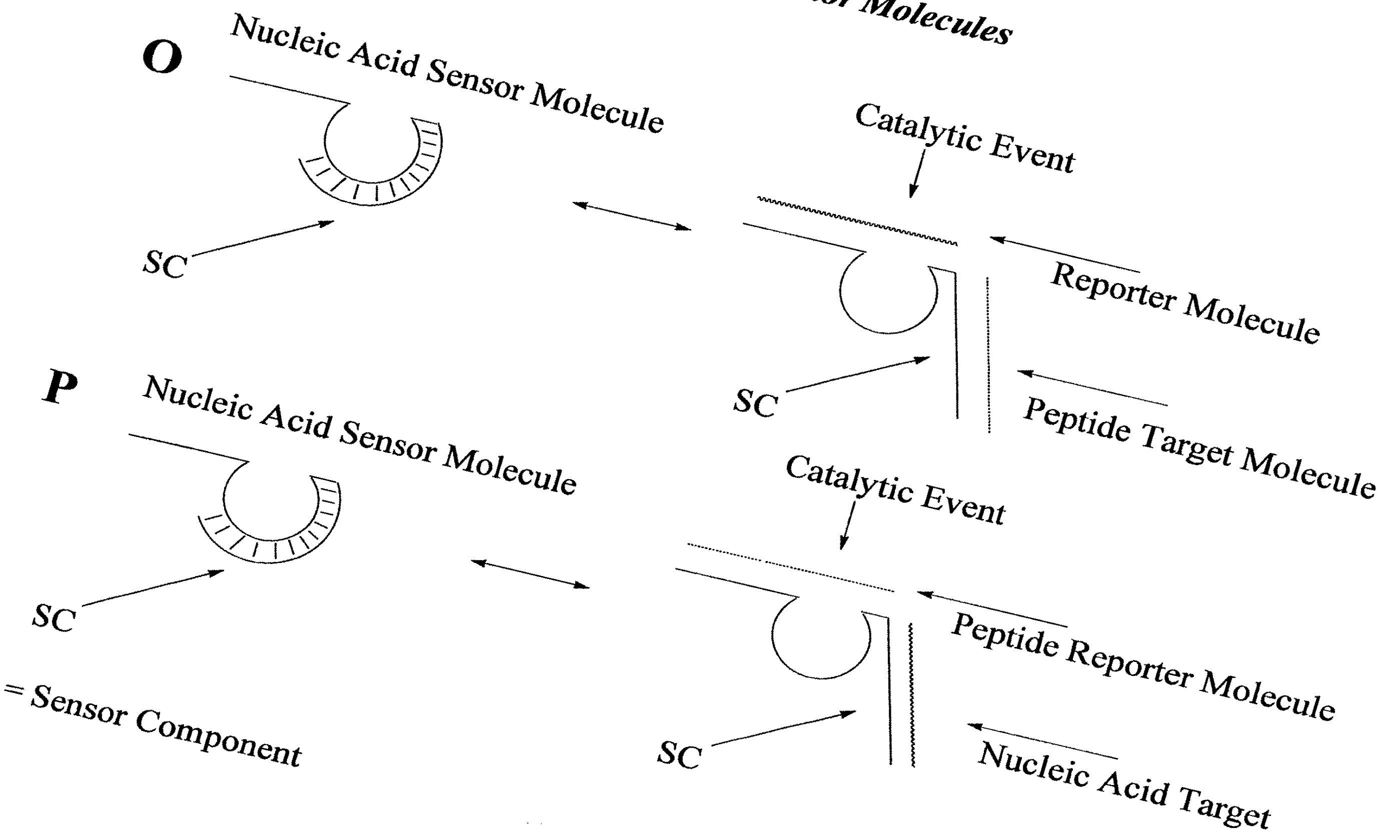


Figure 12: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 13: Examples of Diagnostic Effector Molecules

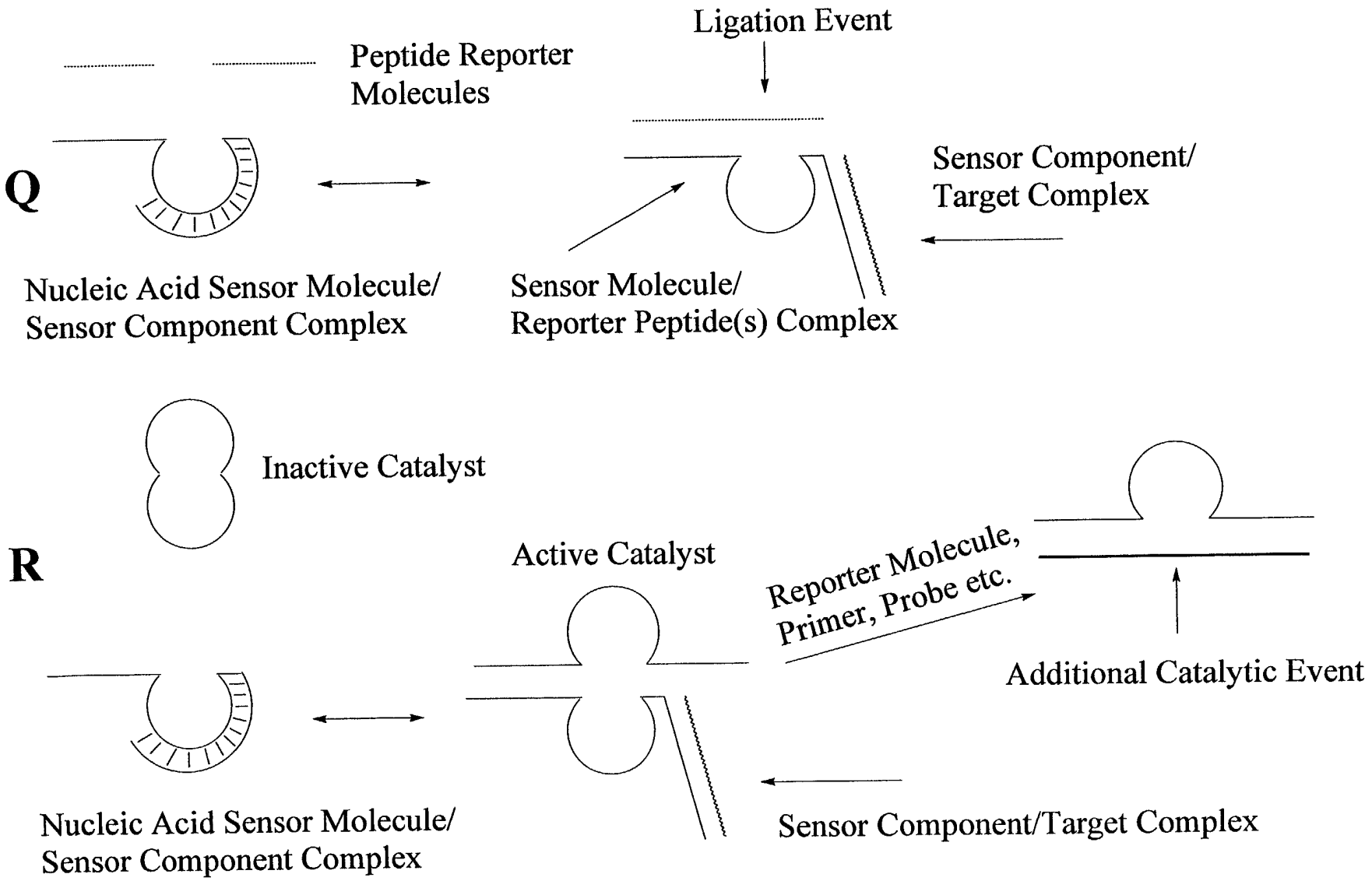


Figure 14: Inherent Amplification of Signal

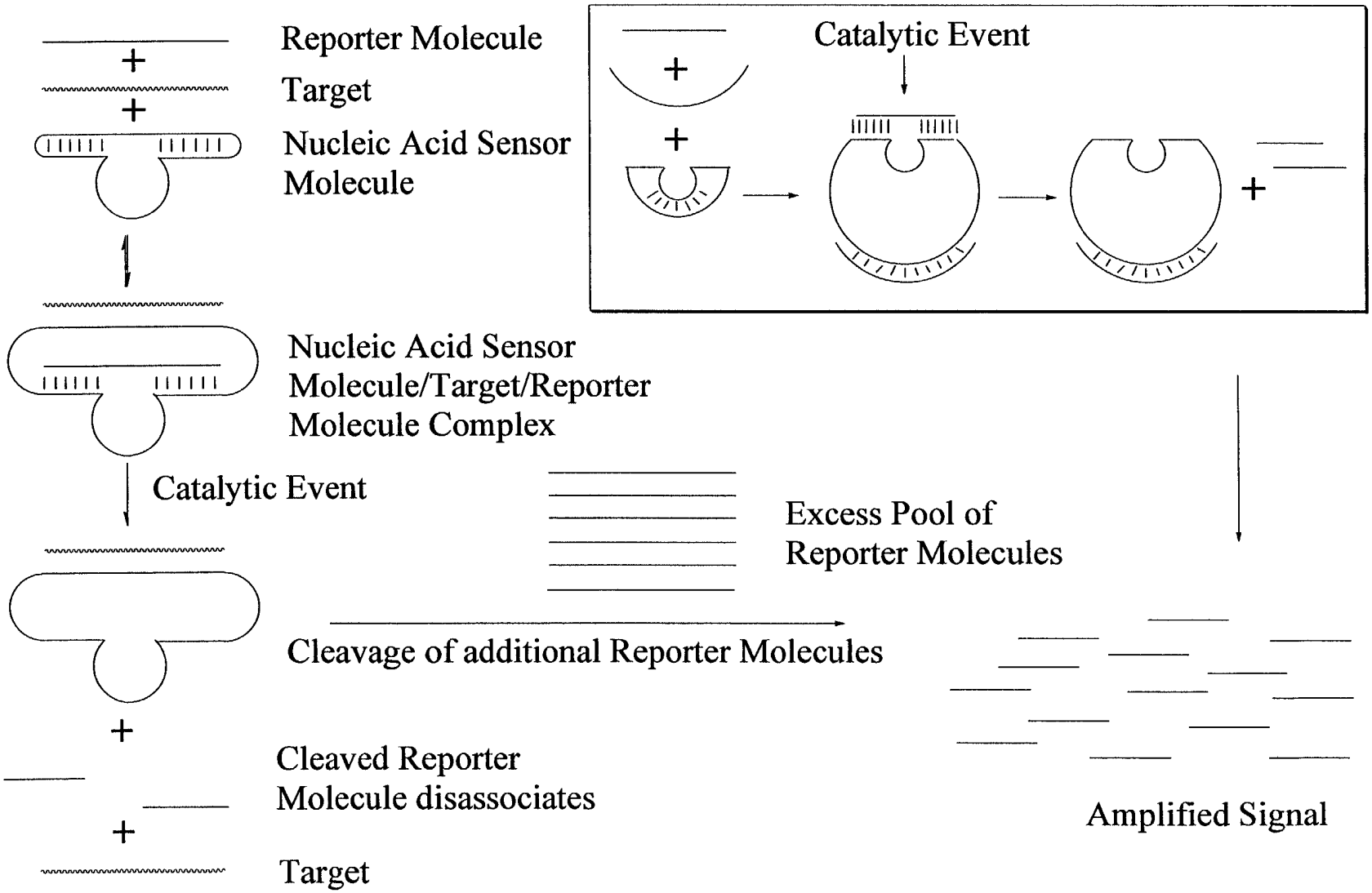


Figure 15: Example of Diagnostic System

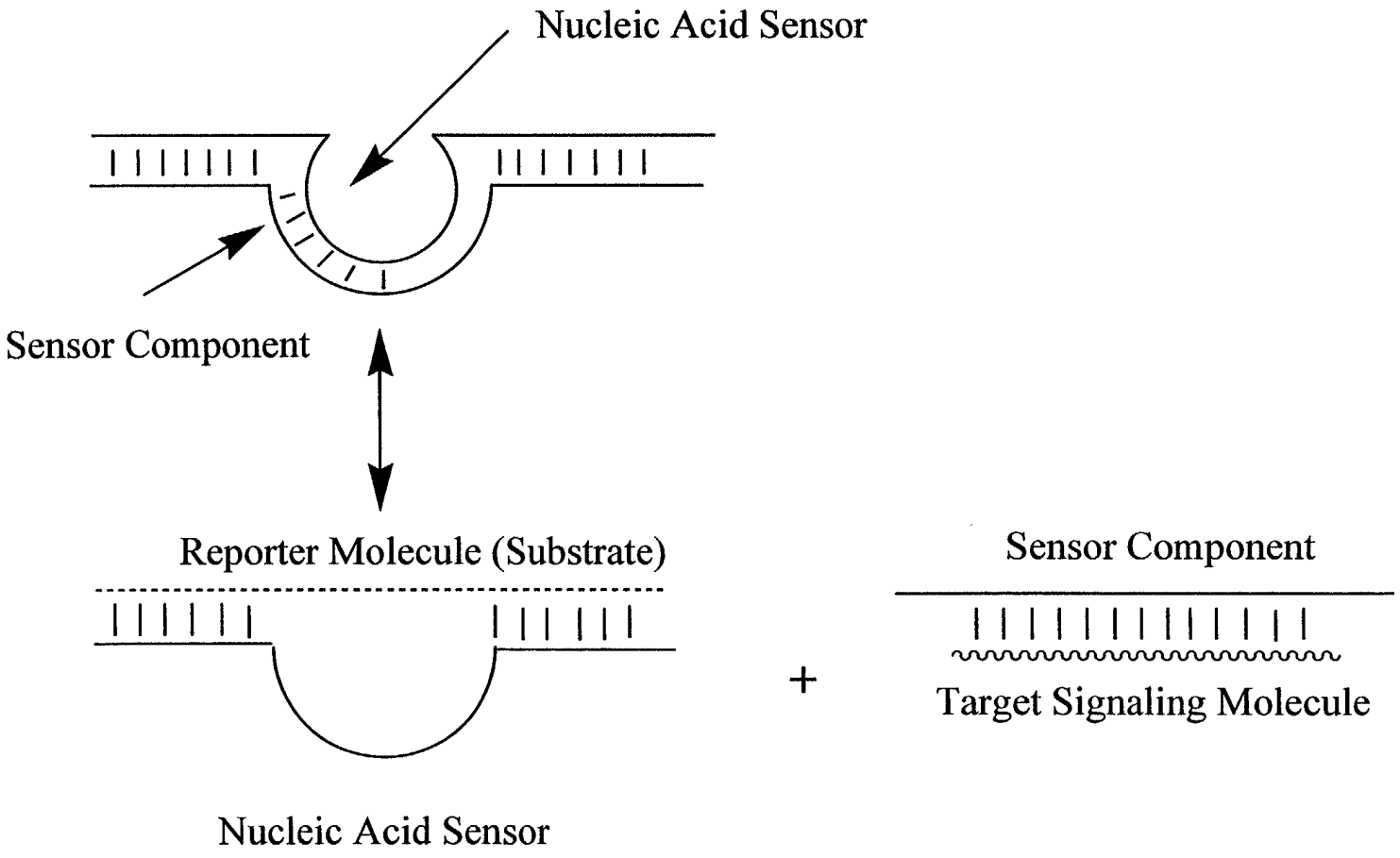


Figure 16: Ribozyme Diagnostic Screen

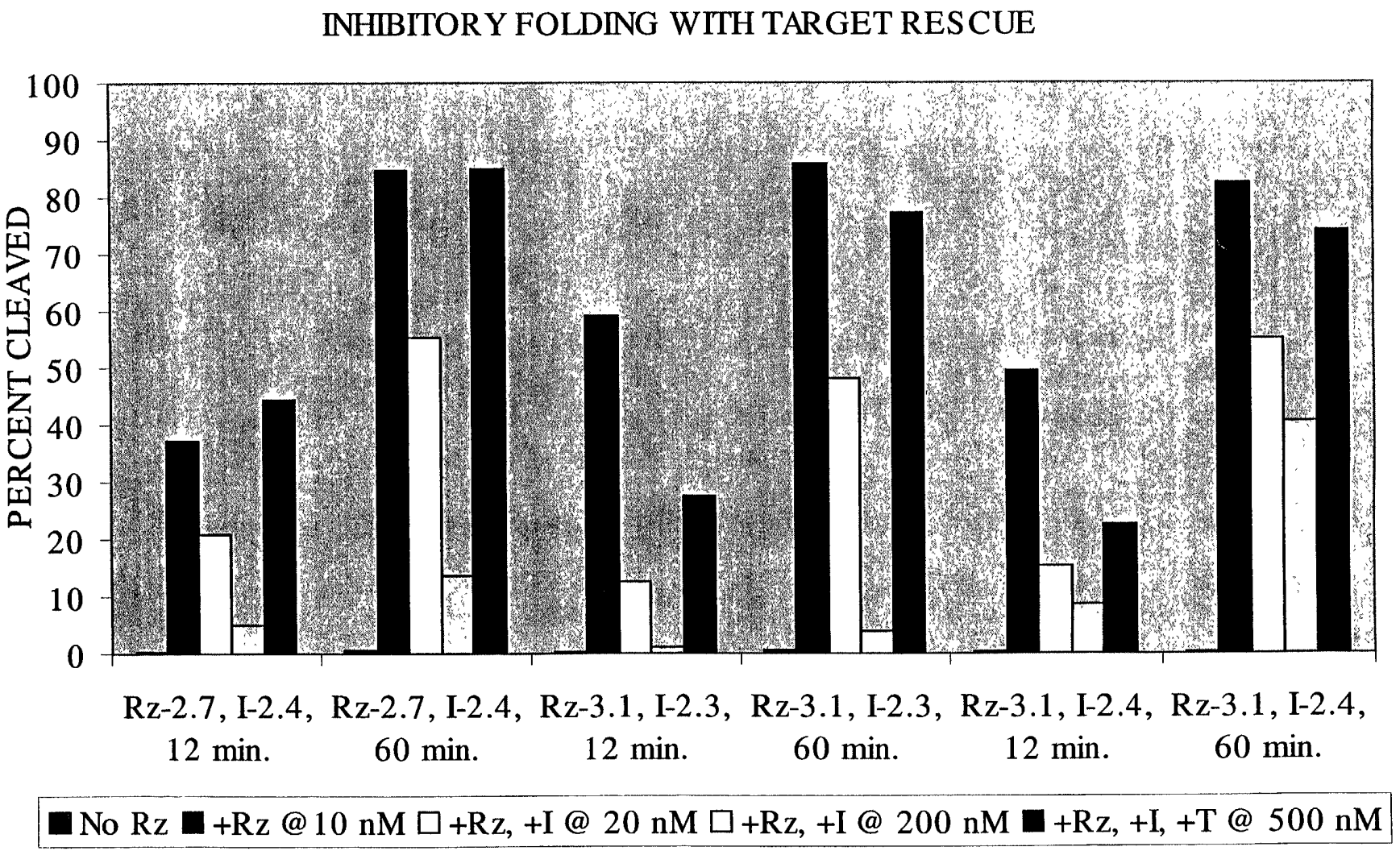


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

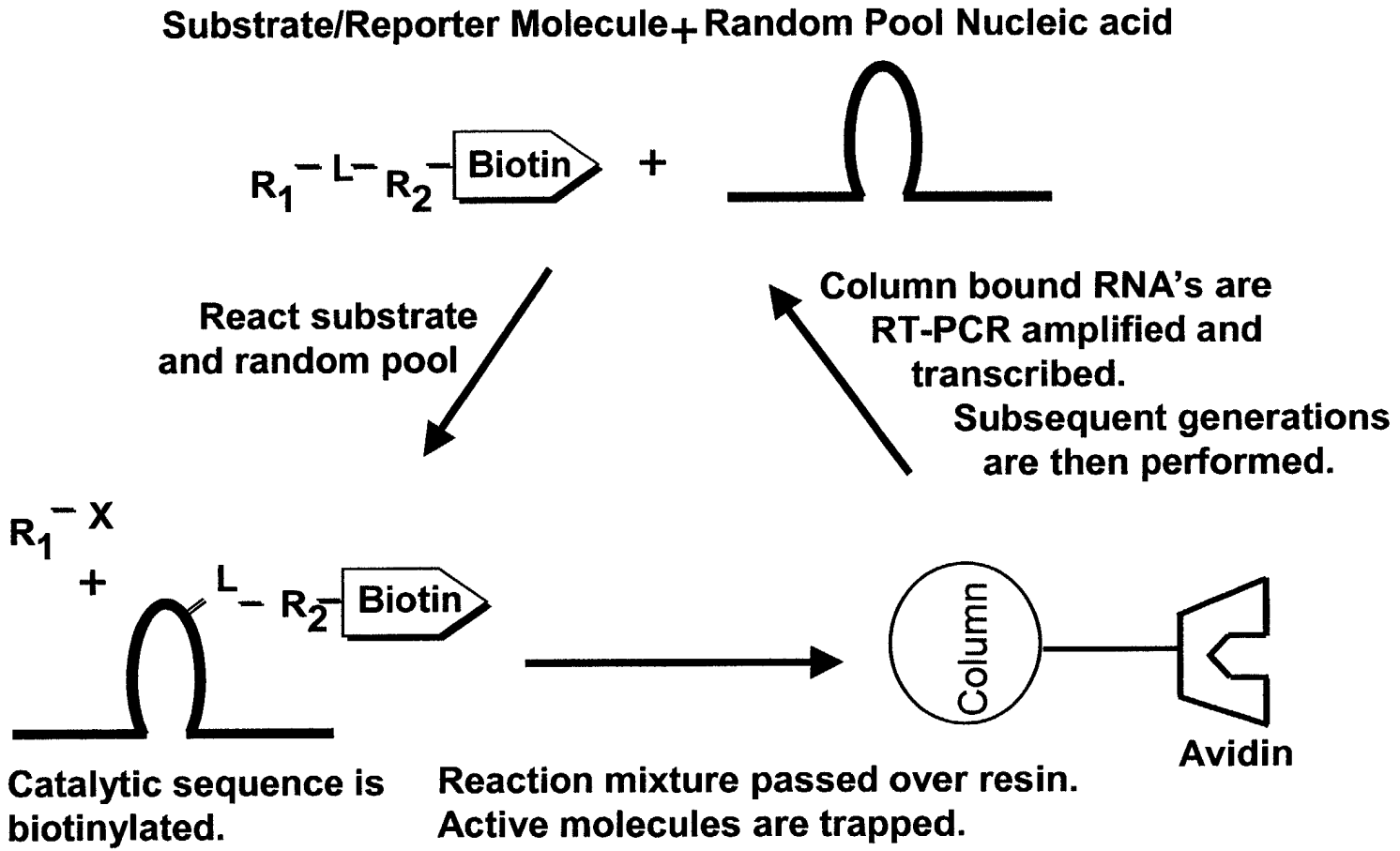


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

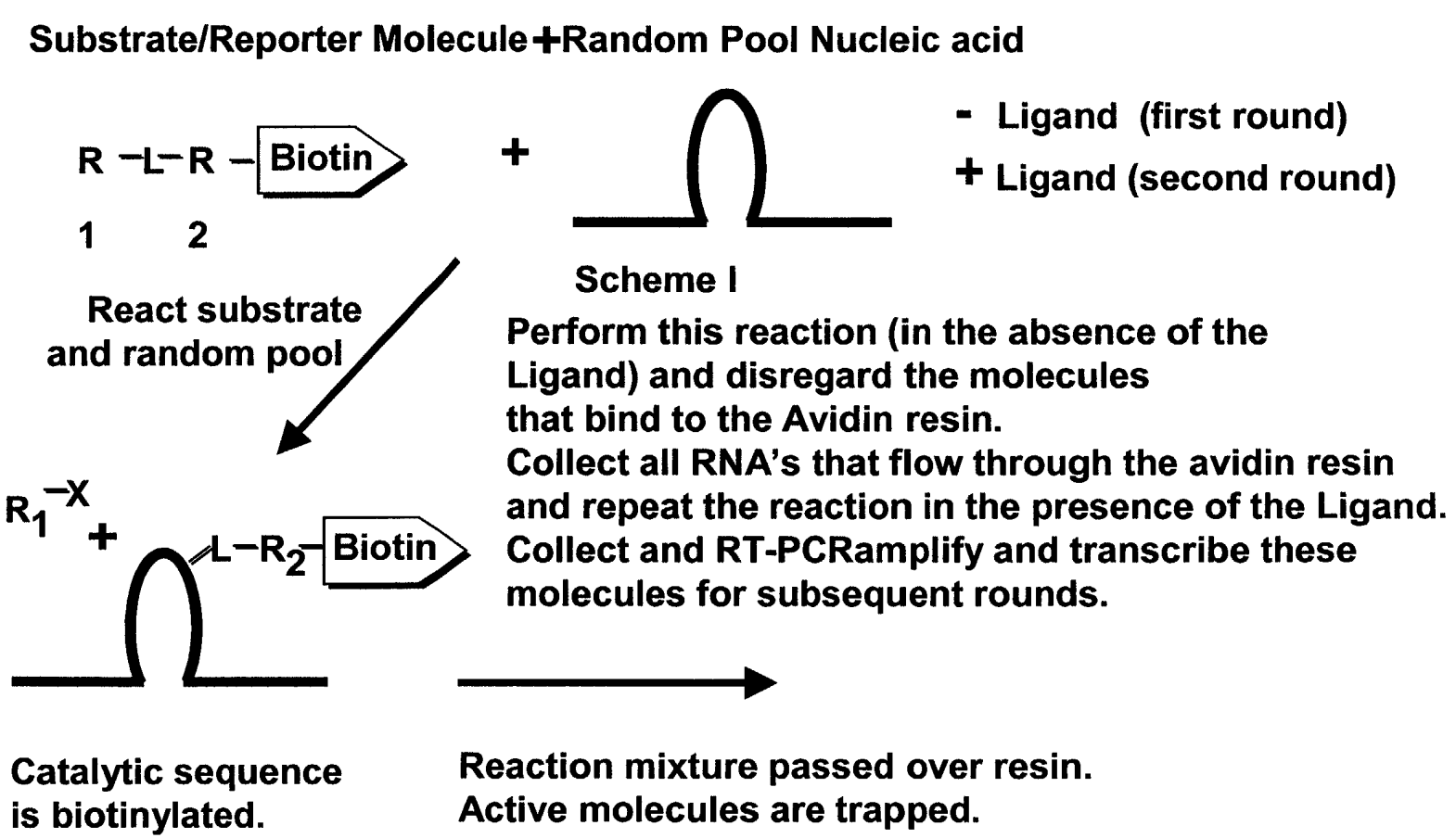


Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules- Ligand dependent

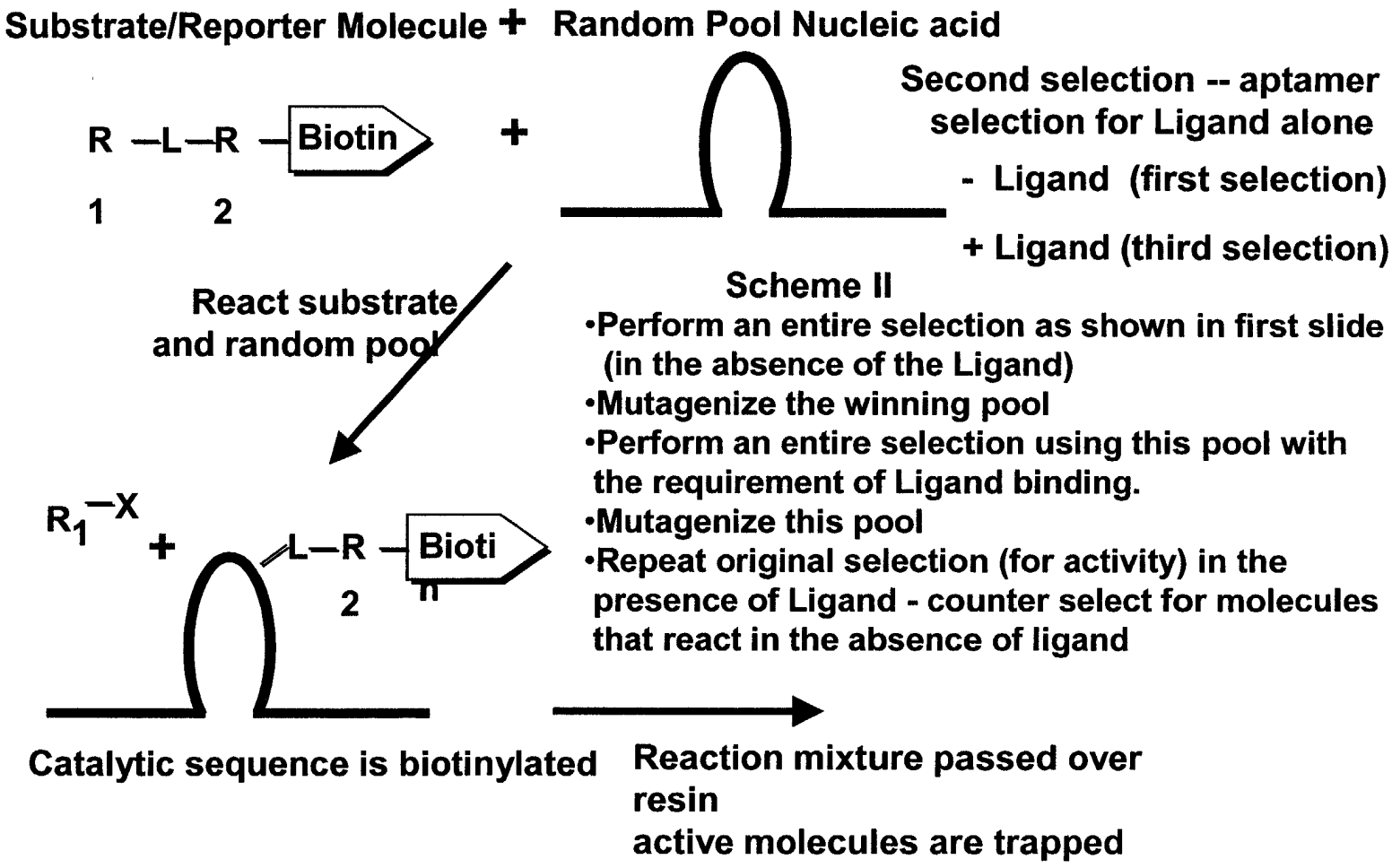


Figure 18a: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

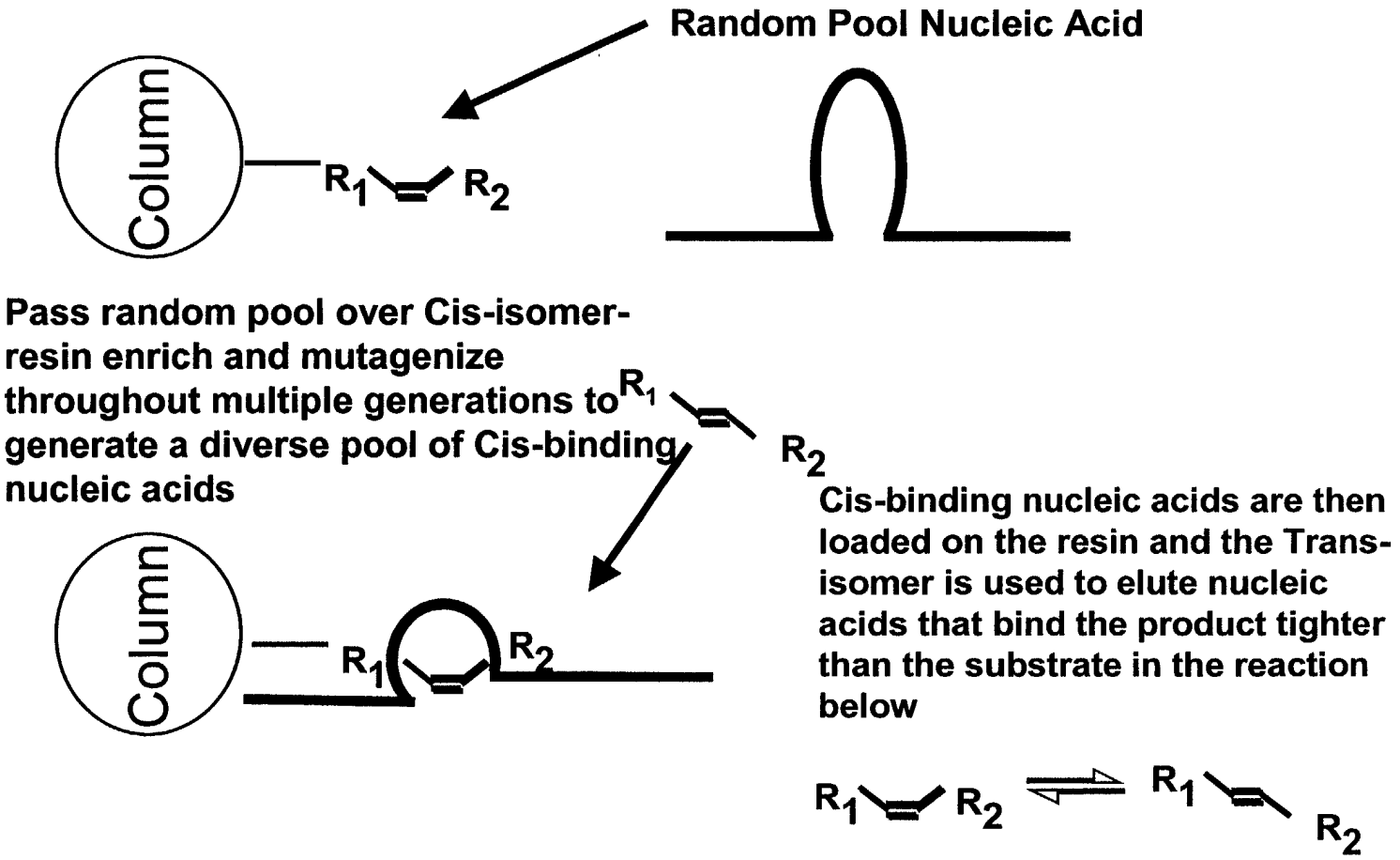
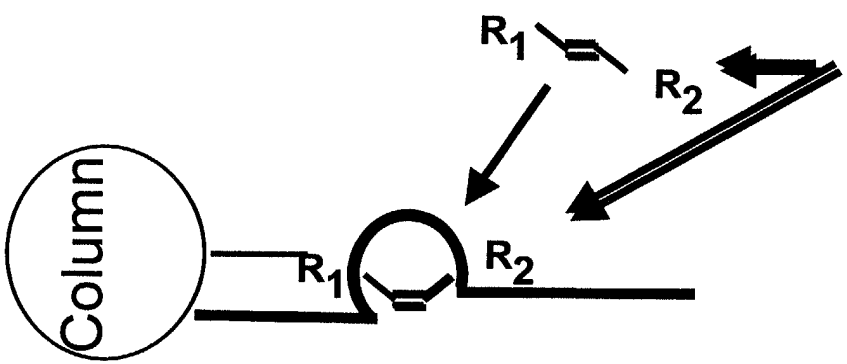


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



The concentration of ligand on the resin and the concentration of eluant ligand can be manipulated in order to bias for enzymatic nucleic acids that prefer one isomer over the other -and can therefore drive the reaction in a desired direction.

E.g. Selection for Cis-isomer at 100 uM - yeild $^{cis}K_d = 100$ Um

Elute with Trans-isomer at 0.1 uM - yield $^{trans}K_d = 0.1$ uM

Isolate catalysts for the reaction below

Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent

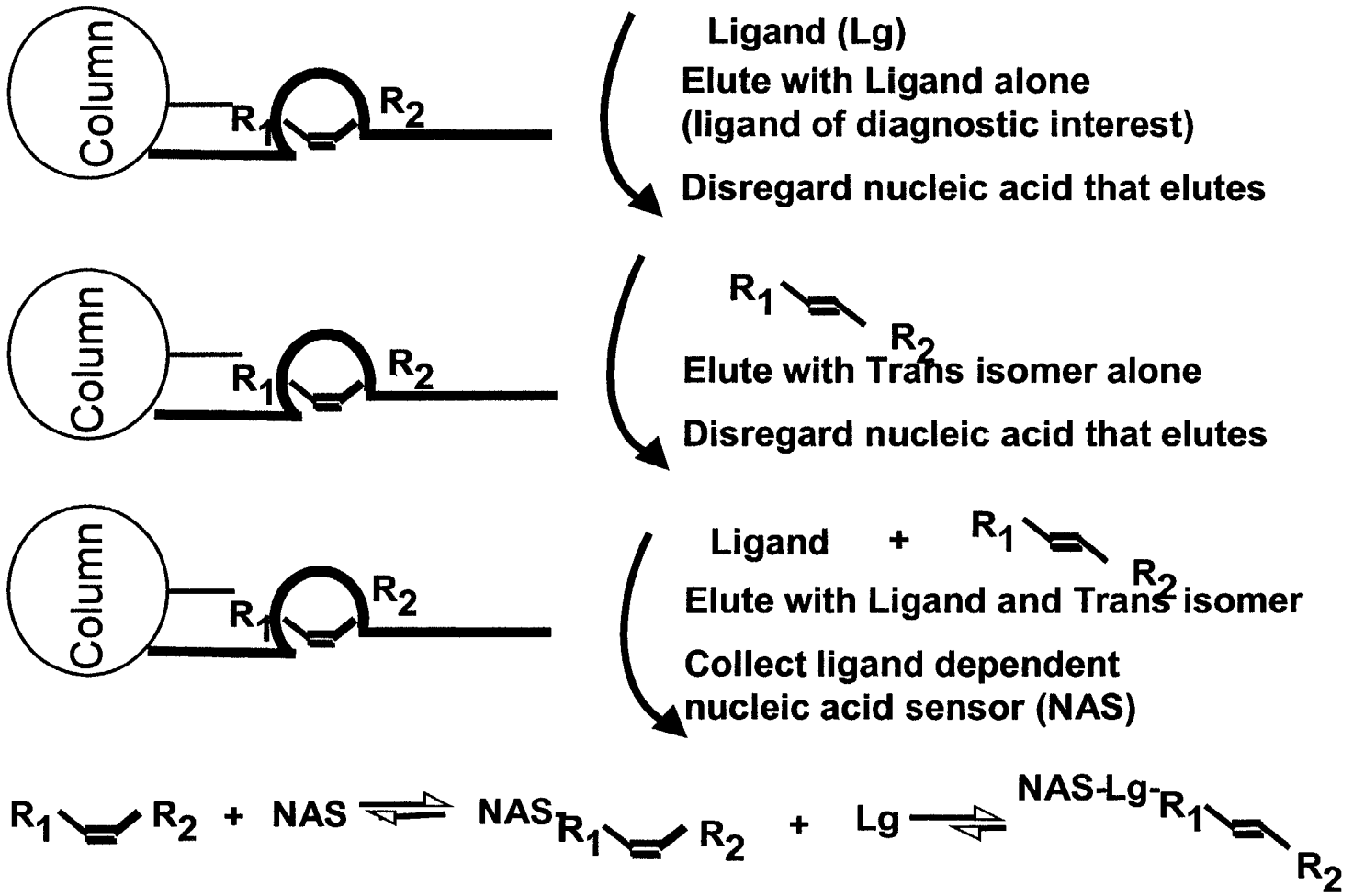
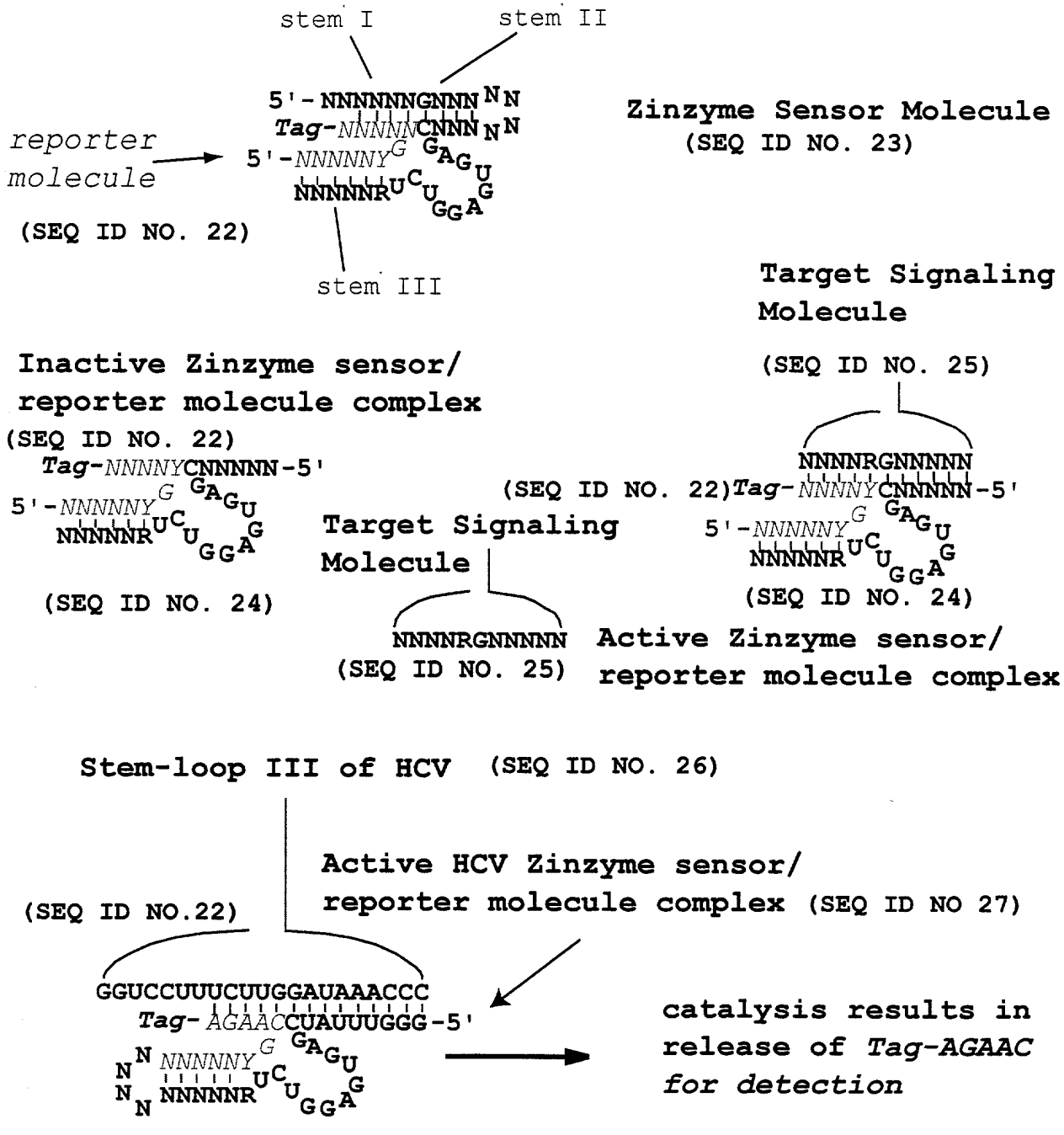
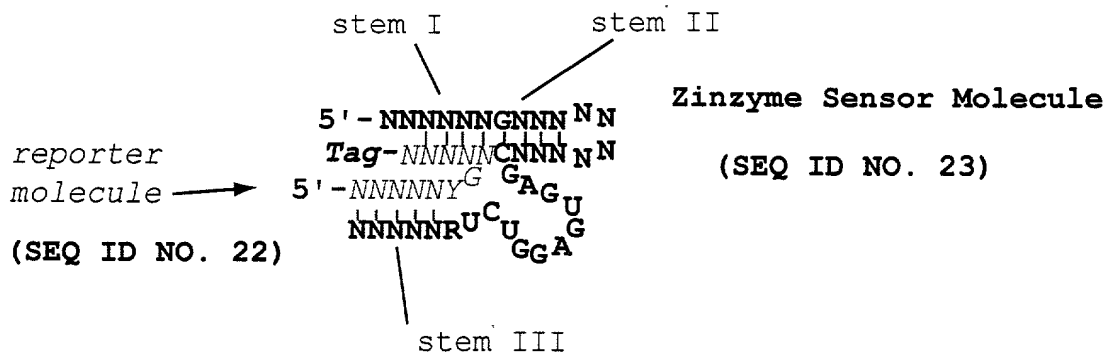


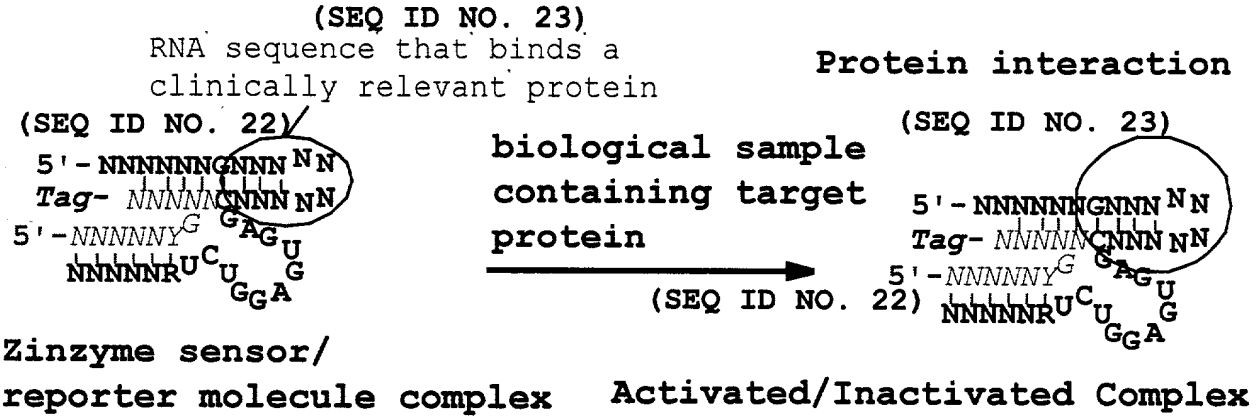
Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid



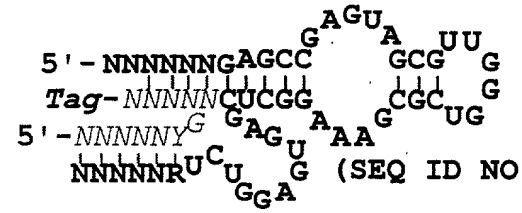
Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

Figure 20: Zinzyme Sensor Molecule for detection of Protein

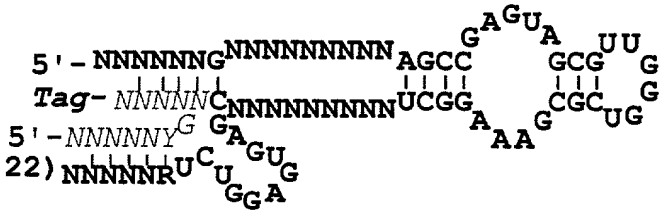




Sensor/reporter complex for detection of HCV core protein

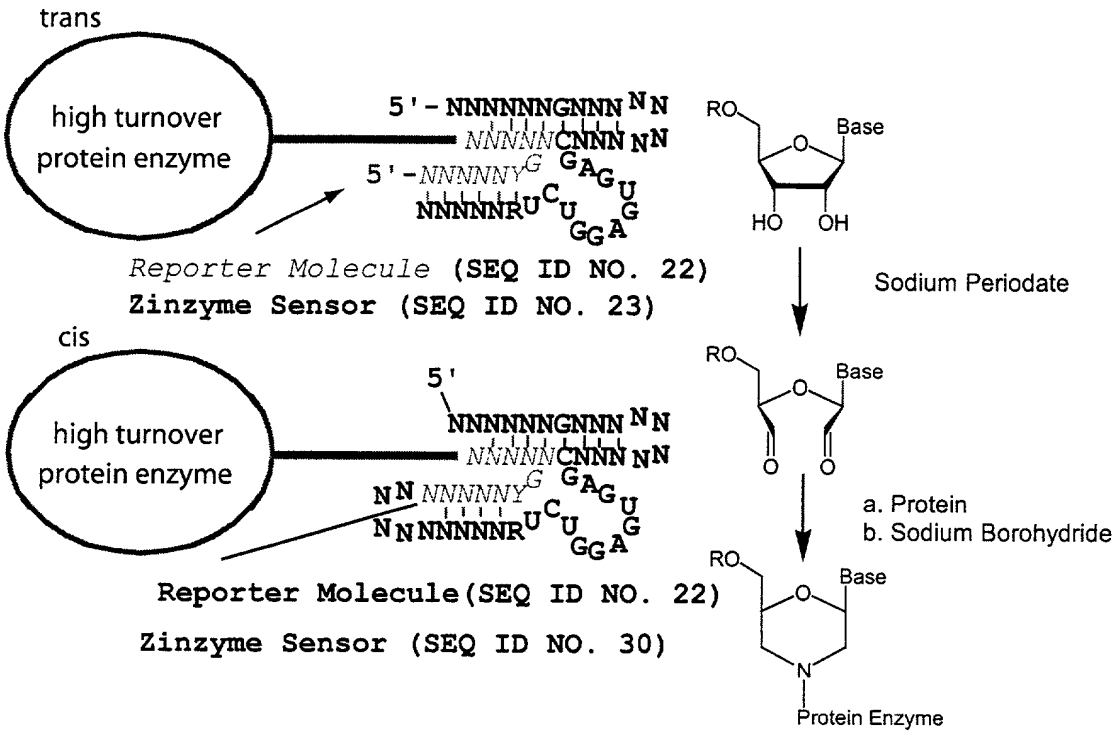


(SEQ ID NO. 22)
HCV Zinzyme sensor with loop IIID of HCV (directs the binding of HCV core protein)
(SEQ ID NO 28)



HCV Zinzyme sensor with loop IIID of HCV connected via randomized linker
(SEQ ID NO 29)

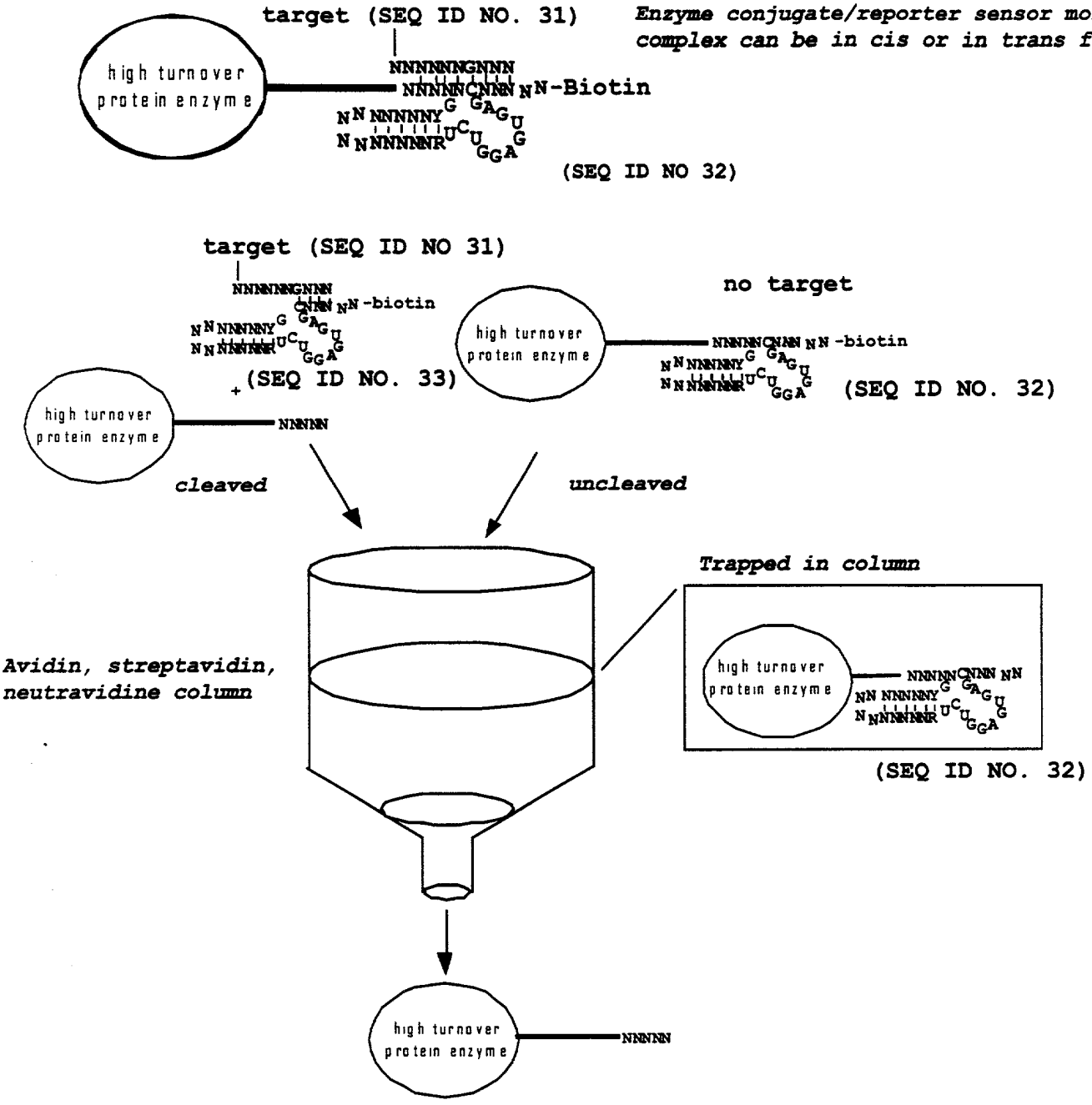
Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.
Protein can be attached via amino linker.
Alternately, R is phorphoramidite moiety for incorporation at 5'-end of oligonucleotide.

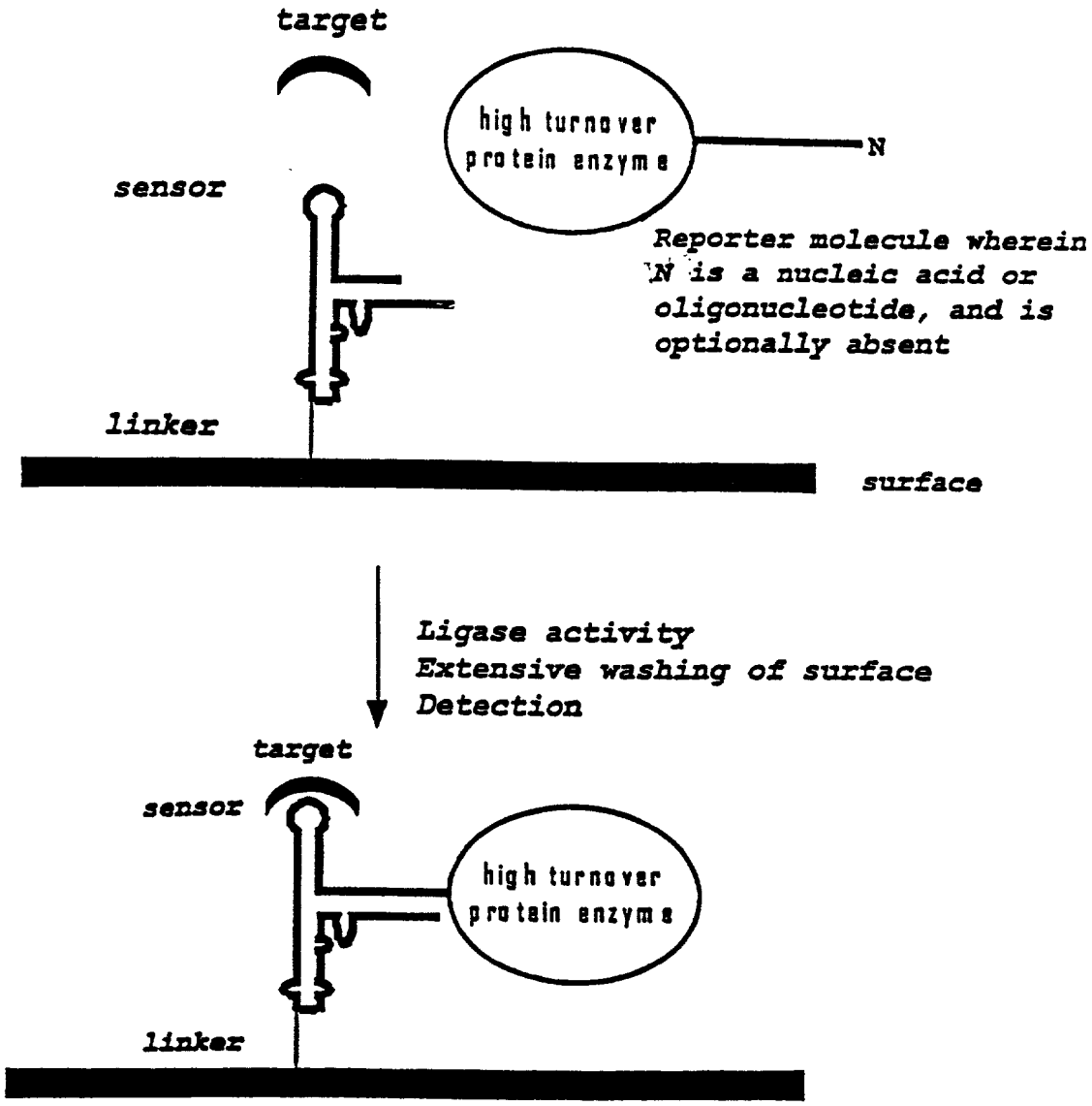
High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

Figure 22: Amplification of signal via use of protein enzyme conjugate



Assay column eluent to determine enzyme activity proportional to sensor molecule activity.

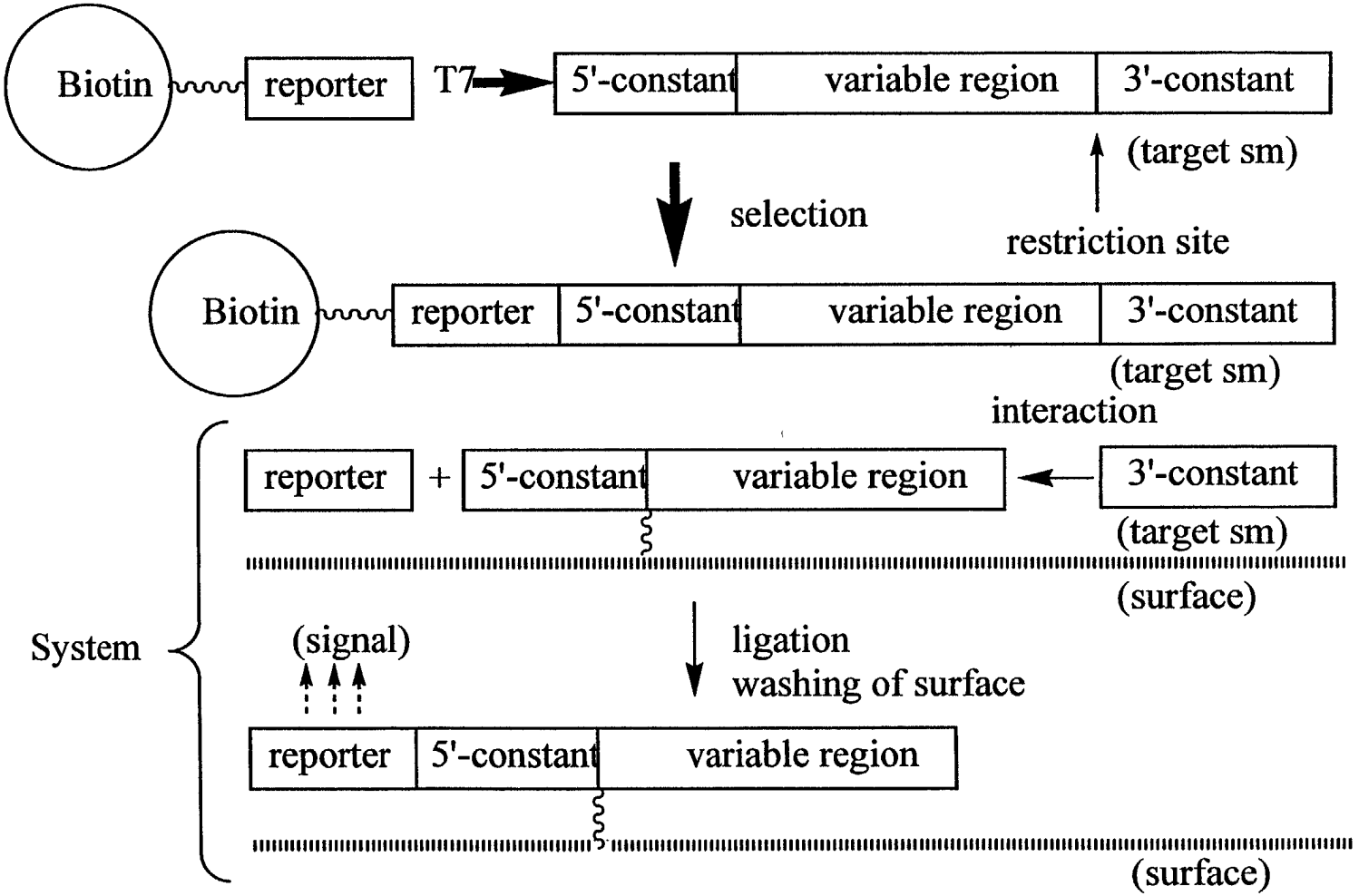
Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

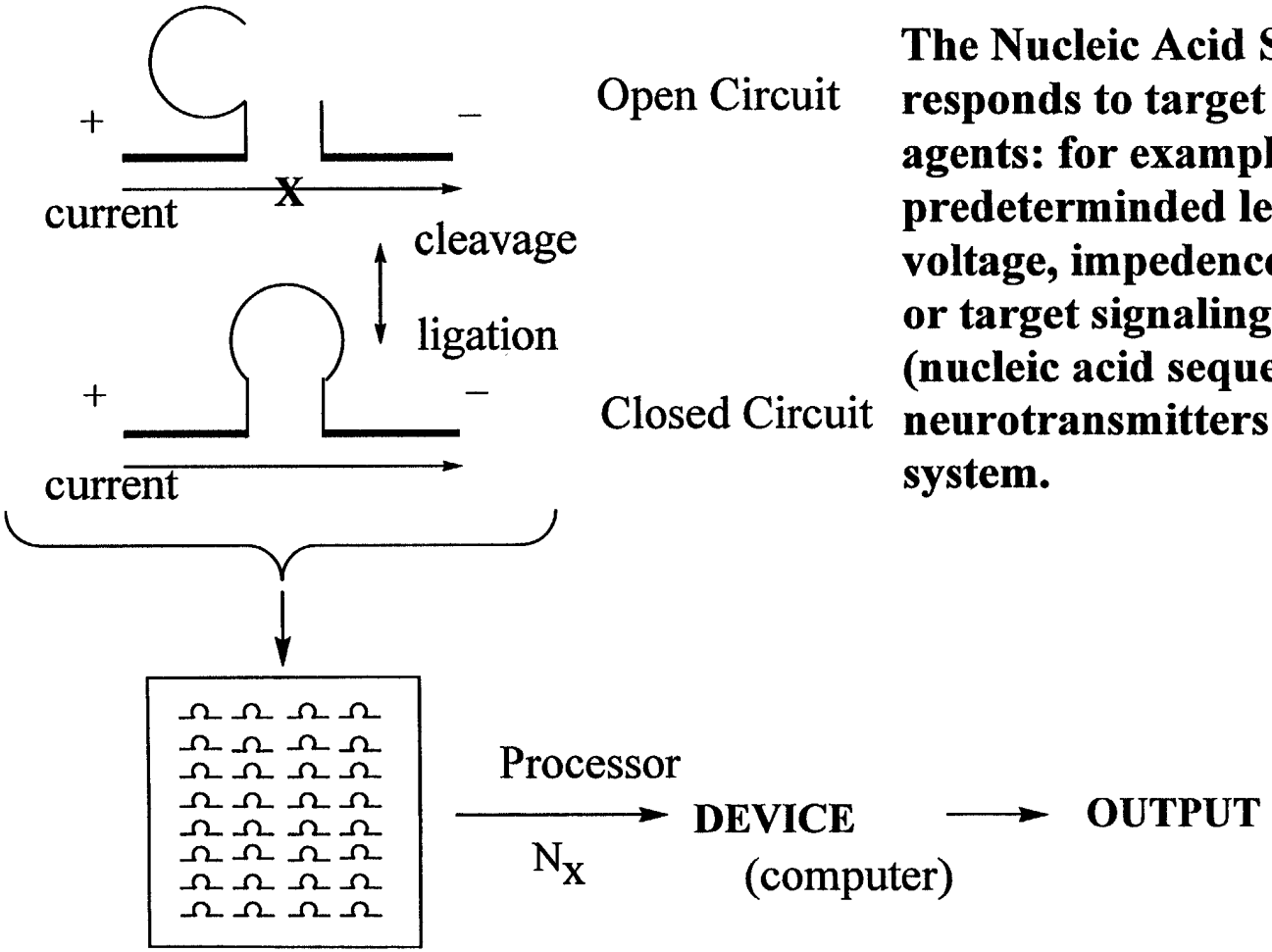
FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity



(sm= signaling molecule)

Figure 25: Nucleic Acid Sensor Molecule-Based Electric Cirucuit



The Nucleic Acid Sensor Circuit responds to target signaling agents: for example predeterminded levels of current, voltage, impedence, or target signaling molecules (nucleic acid sequences, peptides, neurotransmitters etc.) in a system.

Figure 26: Target Inactivation of Zinzyme Sensor Molecule

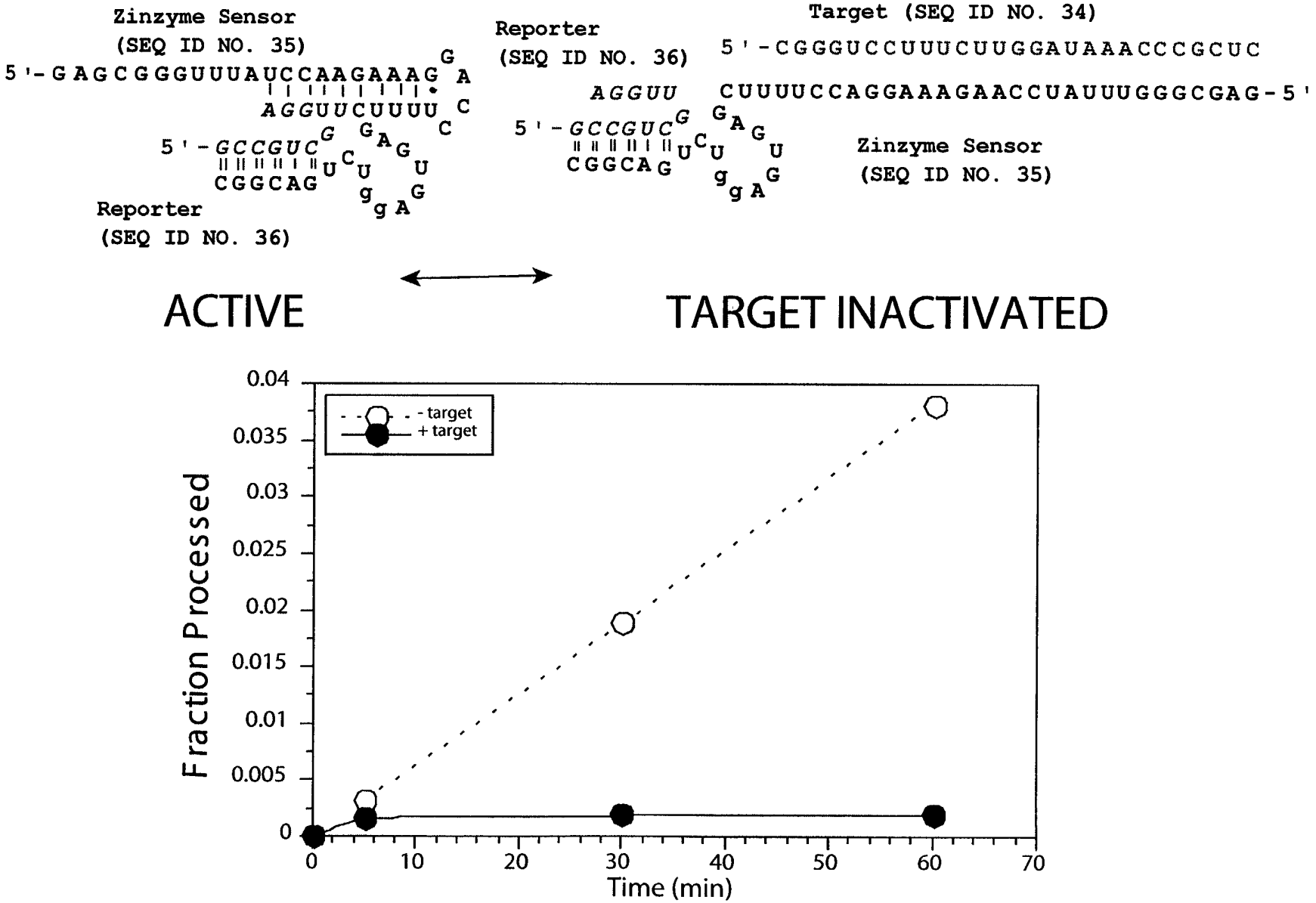


Figure 27: Target Activation of Zinzyme Sensor Molecule

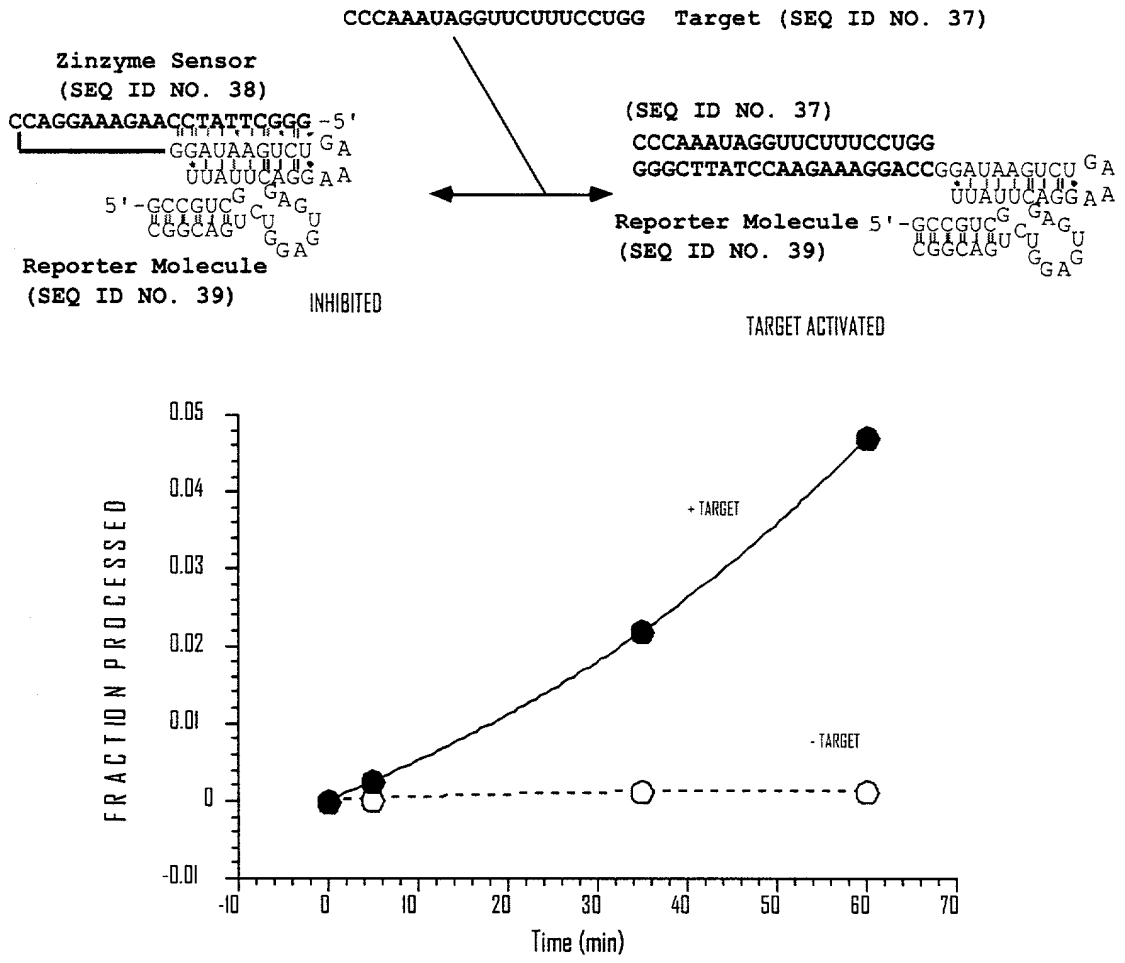
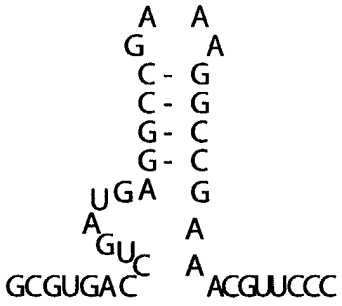
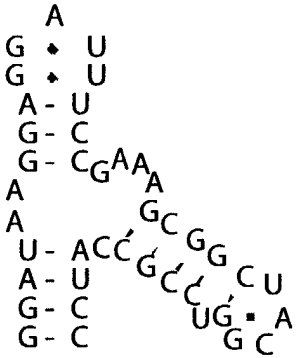


Figure 28: Erk modulated Nucleic Acid Sensor Molecule



HAMMERHEAD (HH)

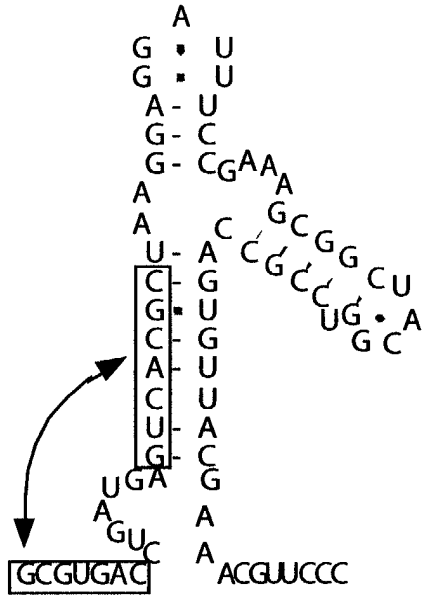
(SEQ ID NO. 40)



ERK SENSOR COMPONENT

K_D=300 pM

(SEQ ID NO. 41)



EHH1.3 sensor molecule

K_D=570 nM

(SEQ ID NO. 42)

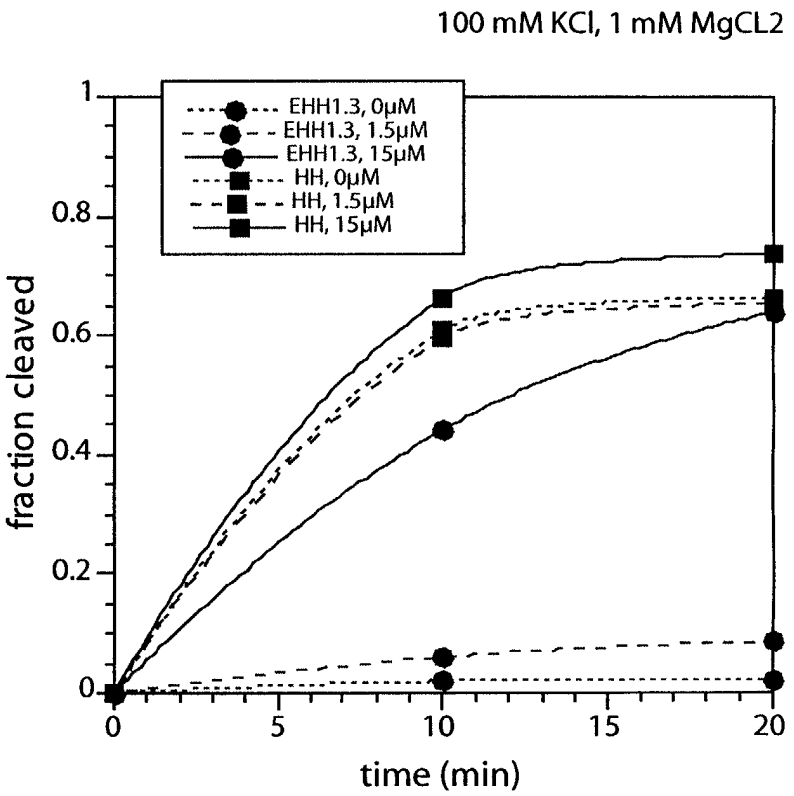


Figure 29: Half-Zinzyme Construct

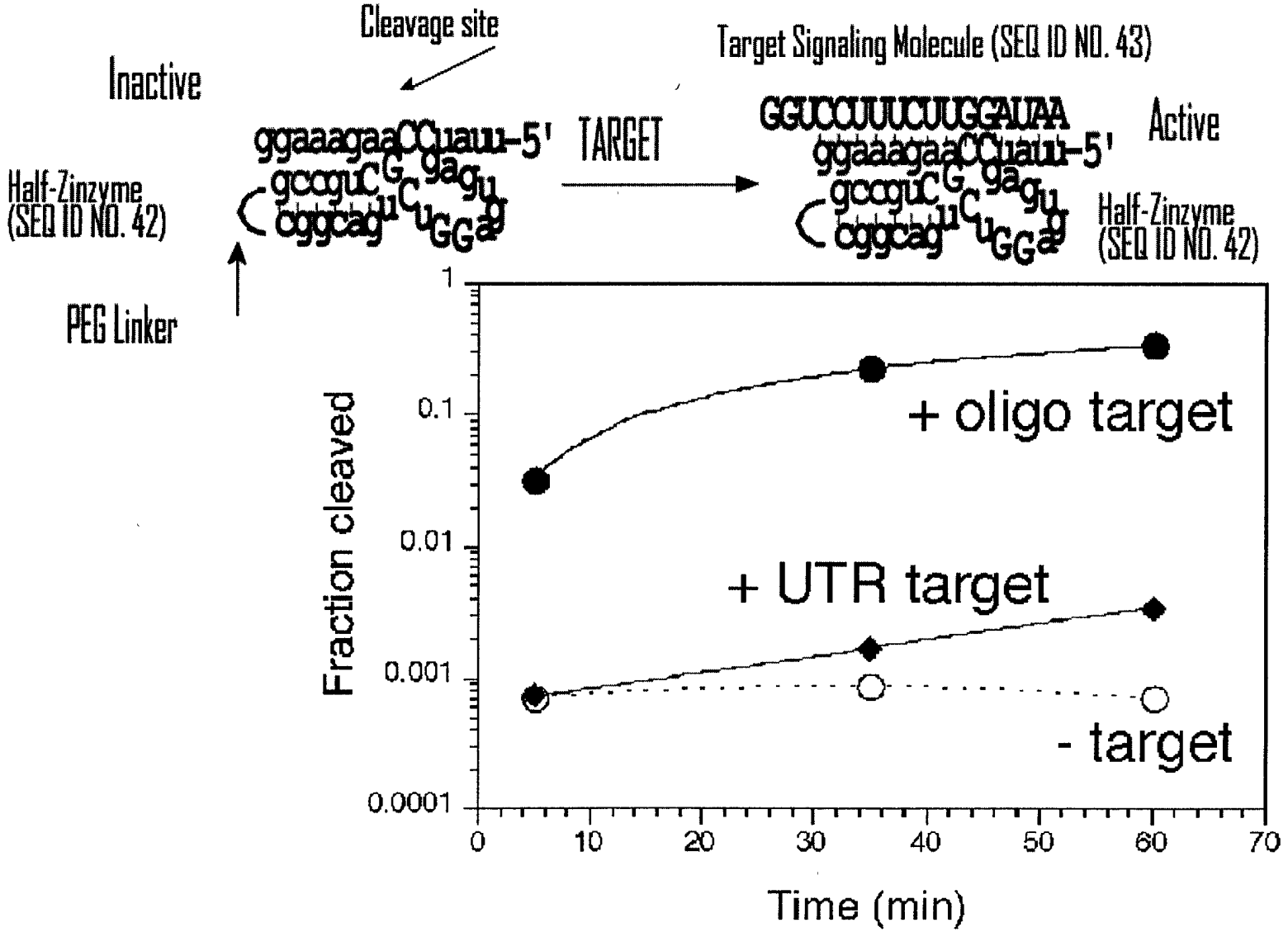
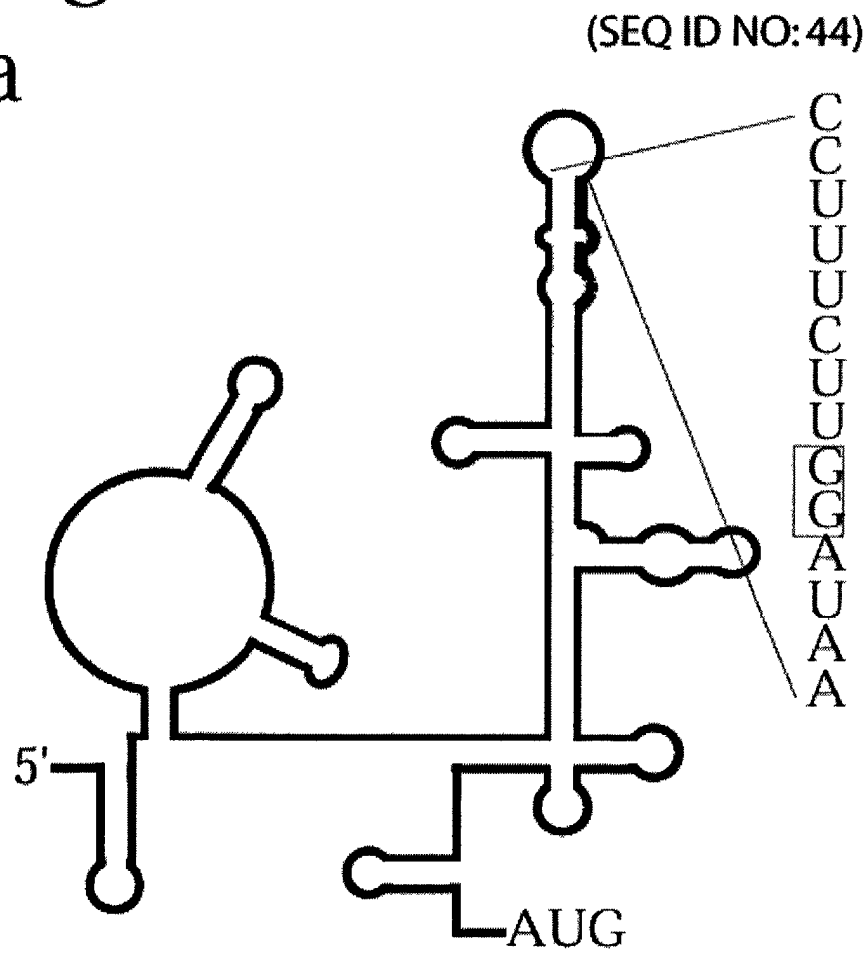


Figure 30



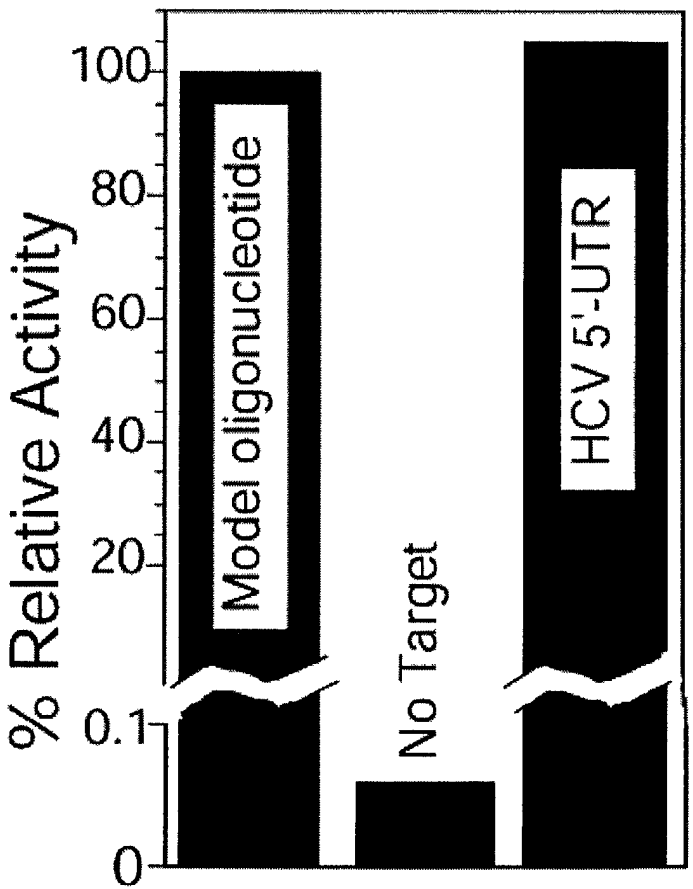


Figure 31

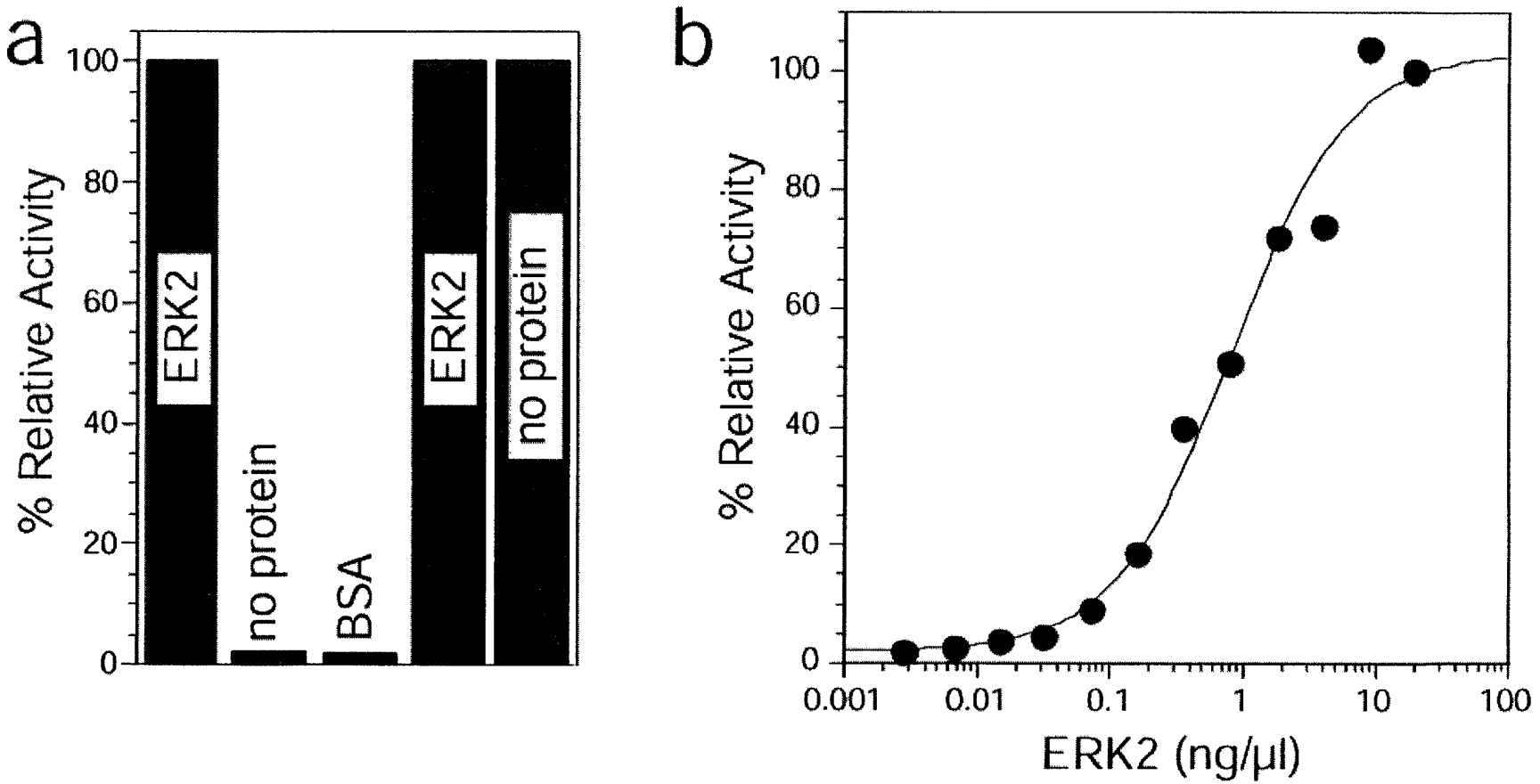


Figure 32

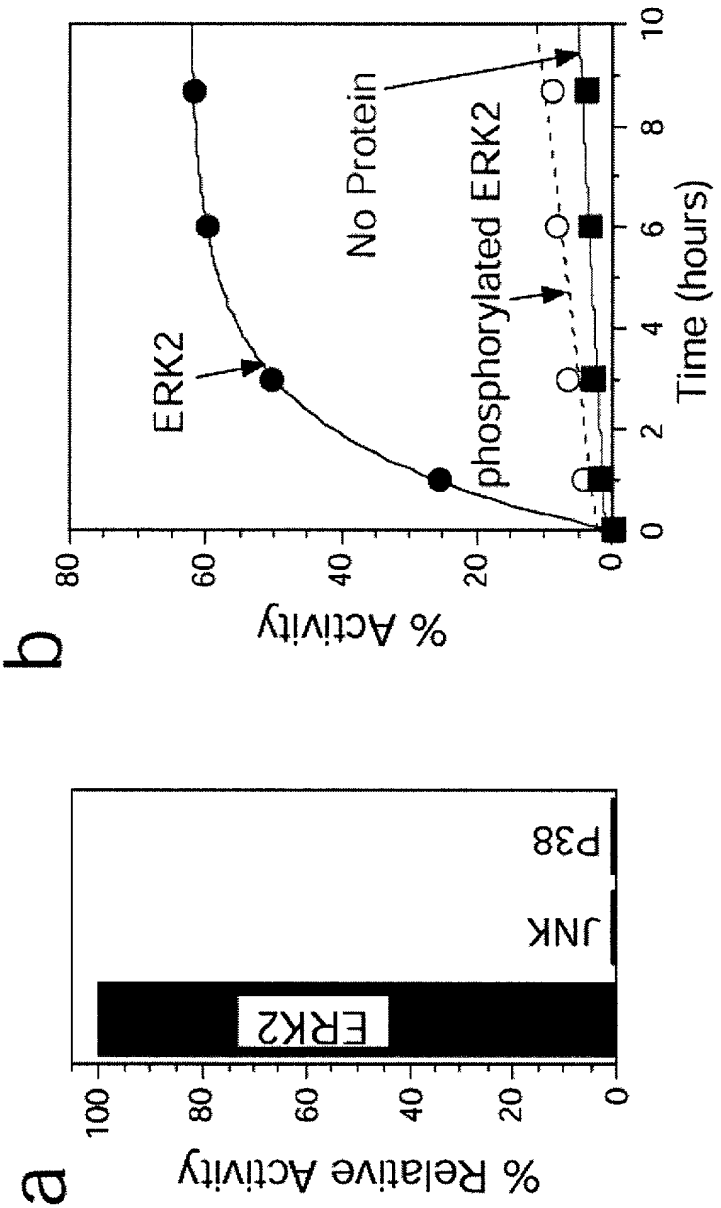

a
% Relative Activity
0
20
40
60
80
100
ERK2
JNK
P38
b
% Activity
0
20
40
60
80
0
2
4
6
8
10
Time (hours)
ERK2
No Protein
phosphorylated ERK2

Figure 33: Halfzyme Ligase

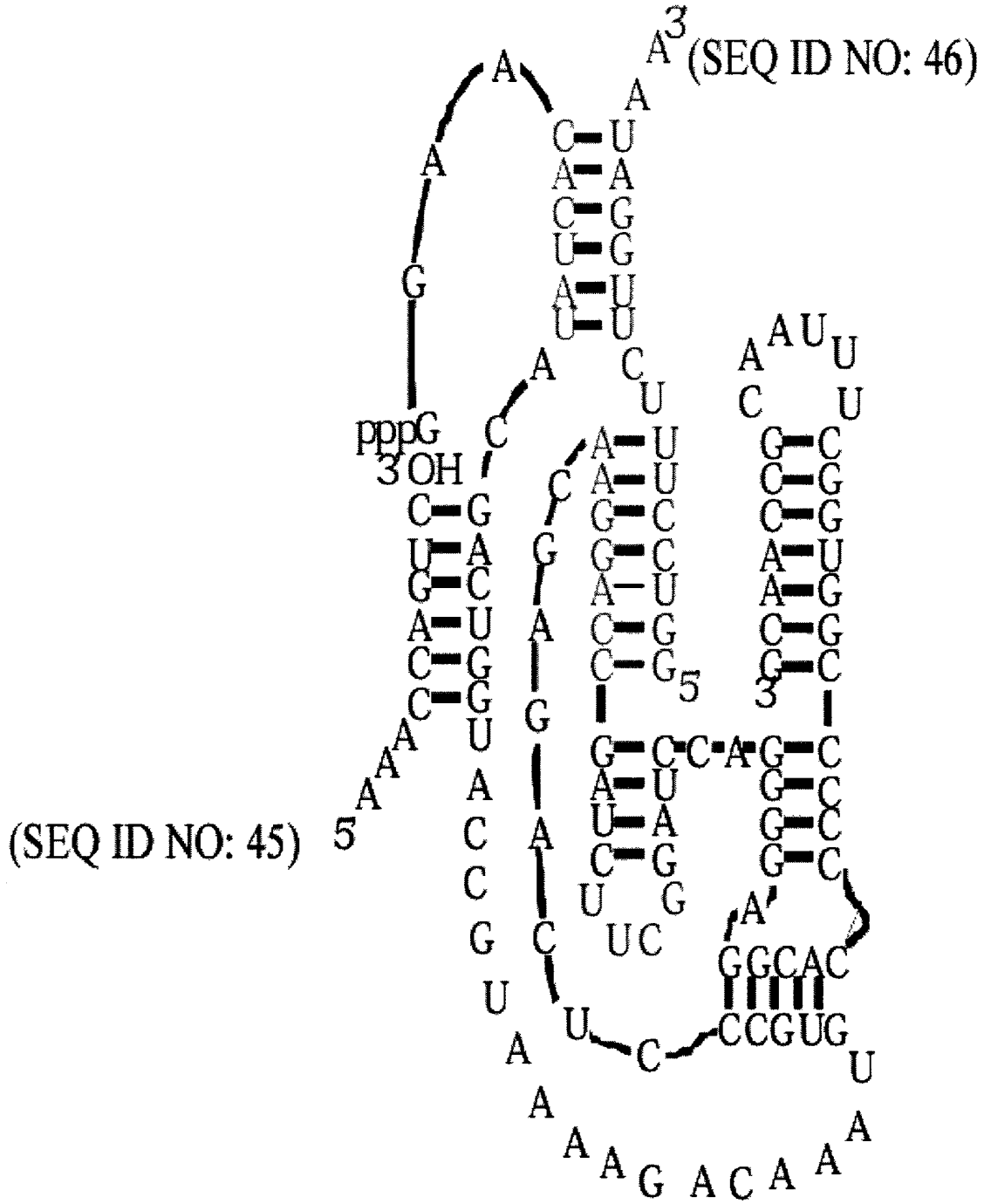


Figure 34: Secondary structure of HCV 5'-UTR

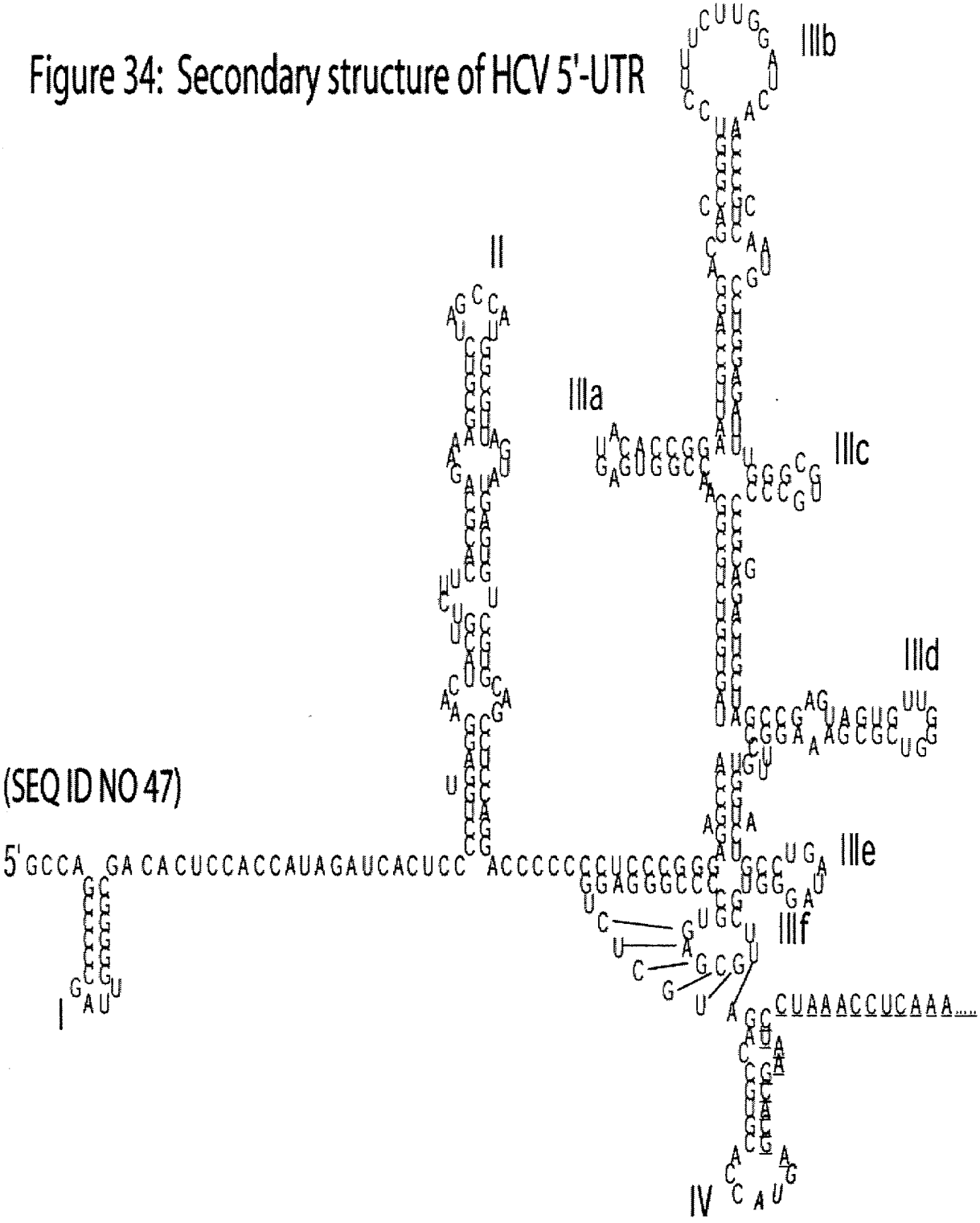
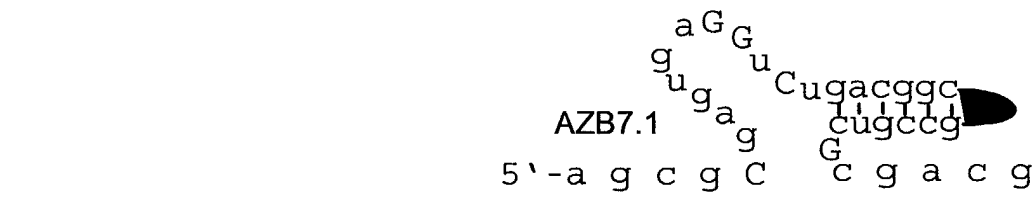


Figure 35
Design of SNP Detection using Halfzyme-AZB7.1



		SEQ ID NO:
	5'-a g c g C c g a c g g g -3'	50
Target HBV 1887(True)=AZB7-GG	3'-T C G C **G** - **G** C T G C C C-5' (SNPT-1)	51
AZB7-AG	3'-T C G C **A** - **G** C T G C C C-5' (SNPT-2)	52
AZB7-TG	3'-T C G C **T** - **G** C T G C C C-5' (SNPT-3)	53
AZB7-CG	3'-T C G C **C** - **G** C T G C C C-5' (SNPT-4)	54
AZB7-GA	3'-T C G C **G** - **A** C T G C C C-5' (SNPT-5)	55
AZB7-GT	3'-T C G C **G** - **T** C T G C C C-5' (SNPT-6)	56
AZB7-GC	3'-T C G C **G** - **C** C T G C C C-5' (SNPT-7)	57
RNA HBV 1433	*3'-U C G C **G** - **G** C U G C C C-5'*	58

Figure 36: Single Nucleotide Polymorphism (SNP) Detection

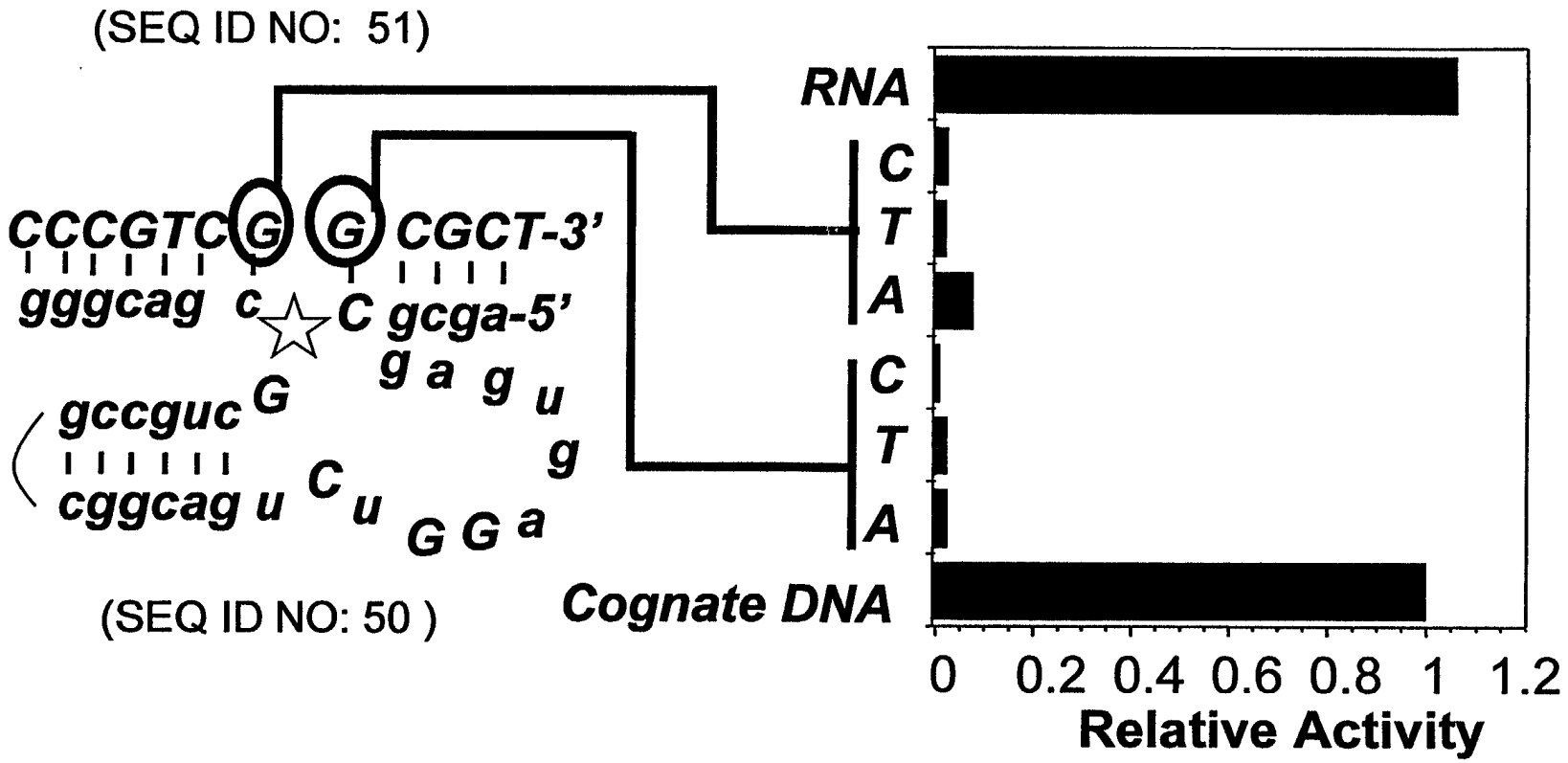
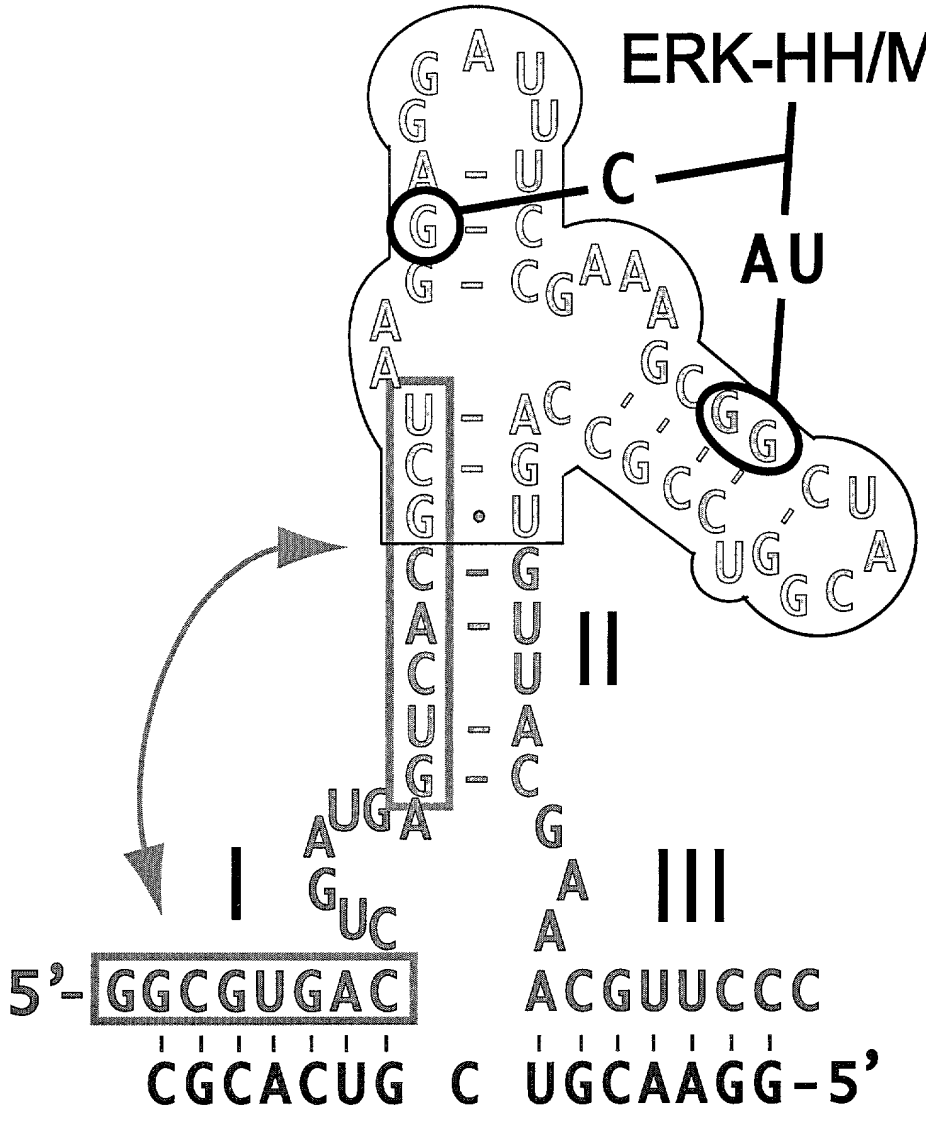


Figure 37A



(SEQ ID NO: 61)

(SEQ ID NO: 62)

Figure 37B

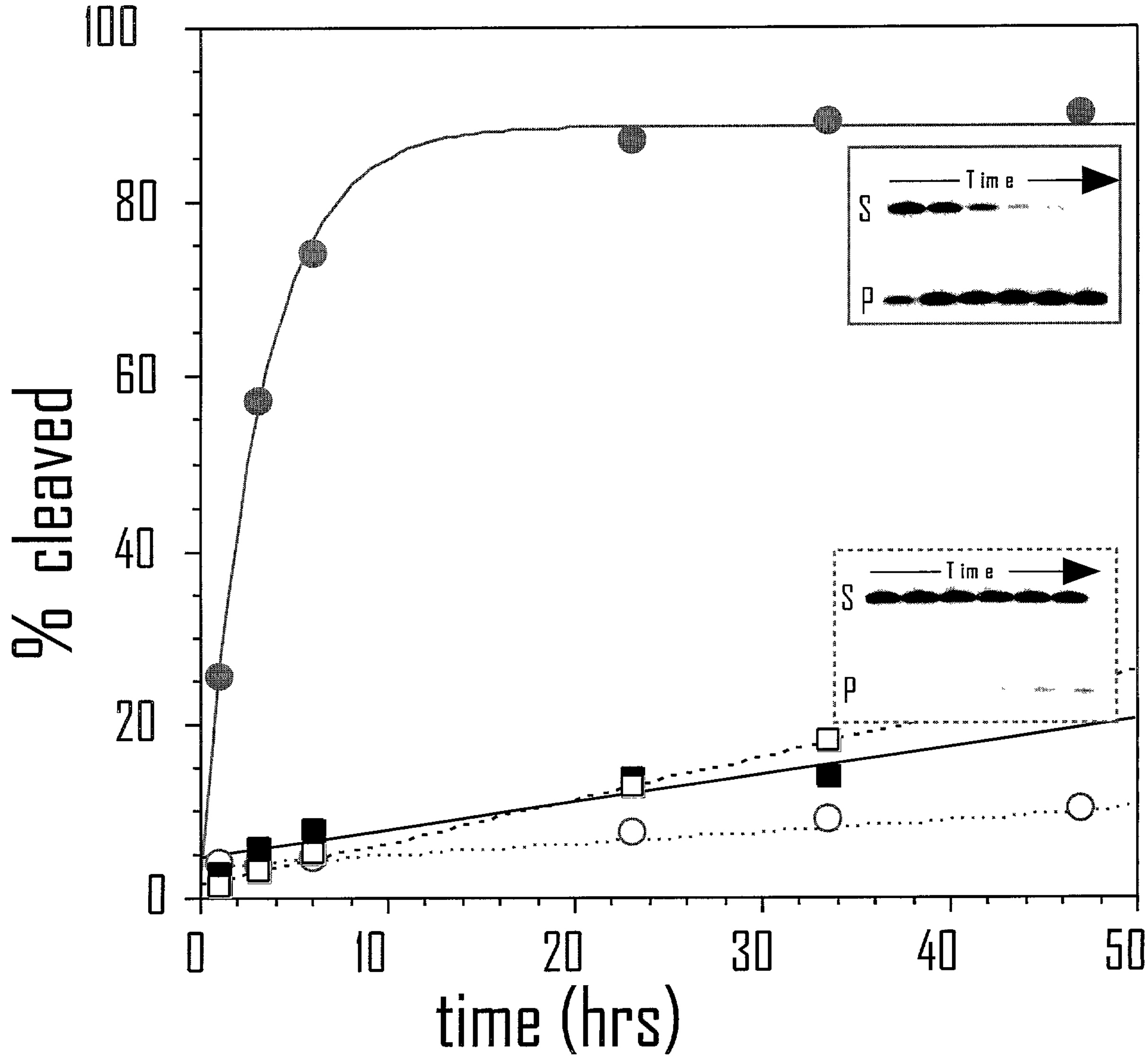


Figure 37C

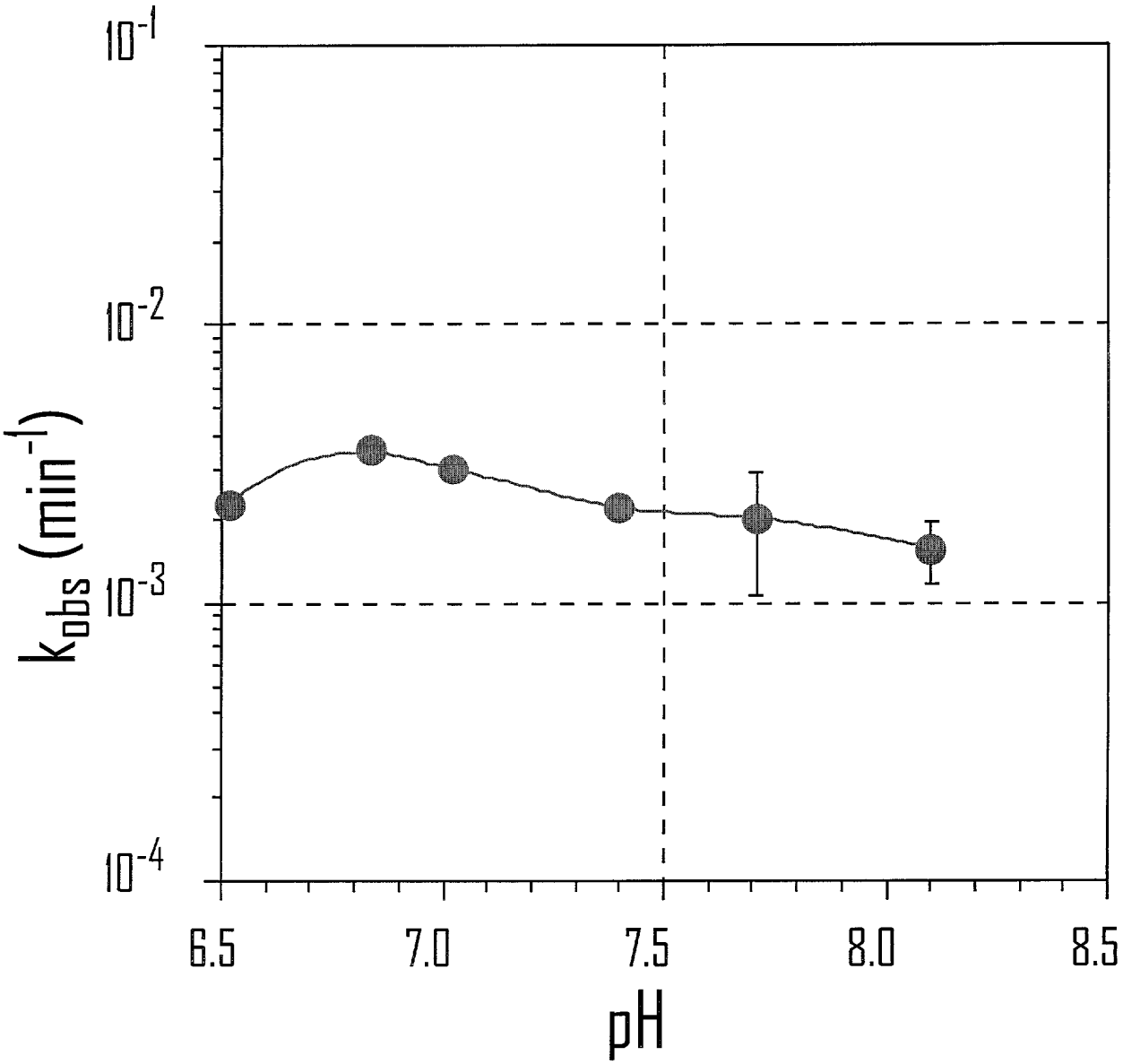


Figure 37D

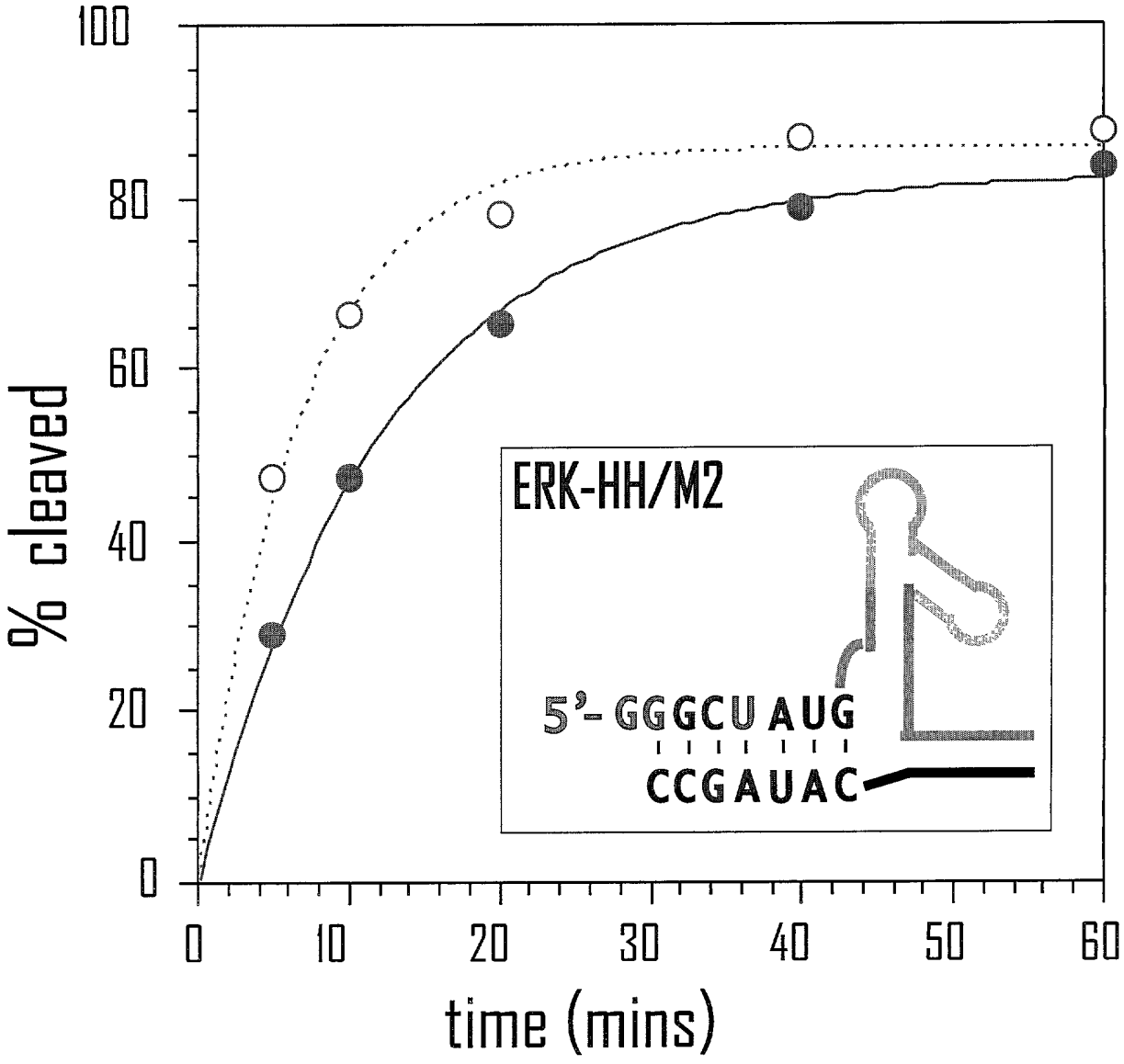

100
80
60
40
20
0
% cleaved
0
10
20
30
40
50
60
time (mins)
ERK-HH/M2
5'- GGGCU AUG
CCGAUAC

Figure 38

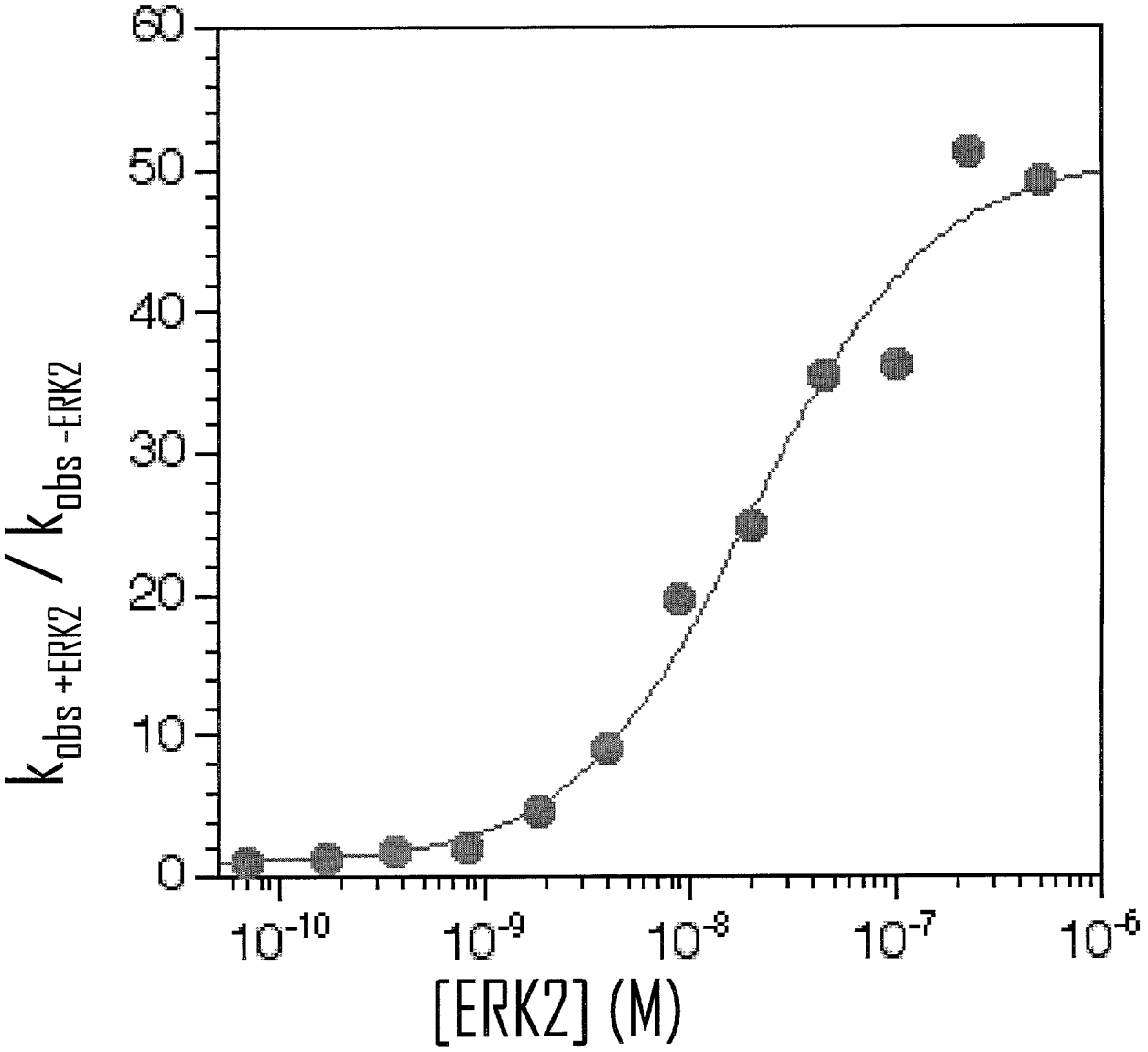

60
50
40
30
20
10
0
$k_{obs\ +ERK2}$ / $k_{obs\ -ERK2}$
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
[ERK2] (M)

Figure 39A

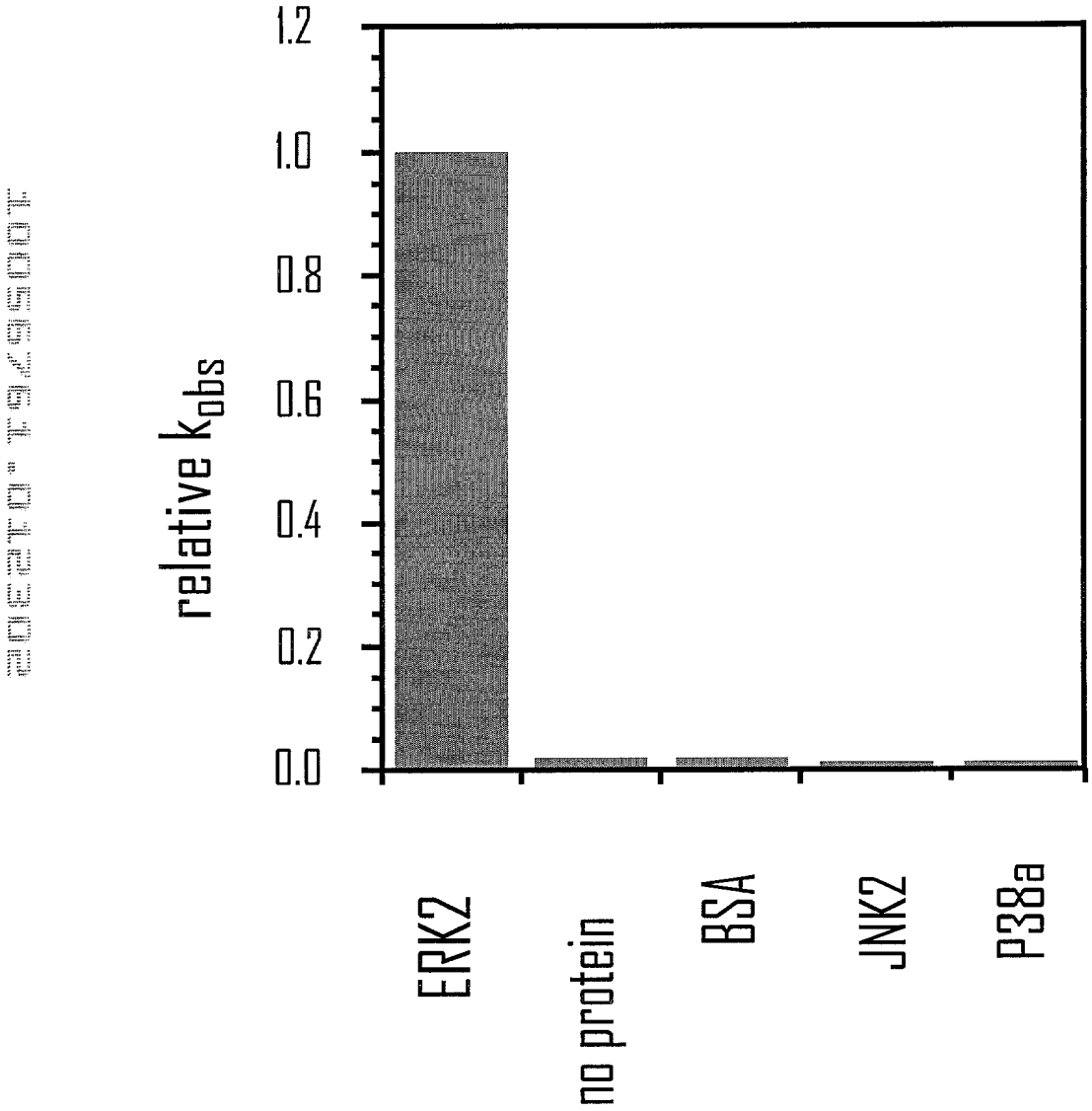

relative k_{obs}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ERK2
no protein
BSA
JNK2
P38a

Figure 39B

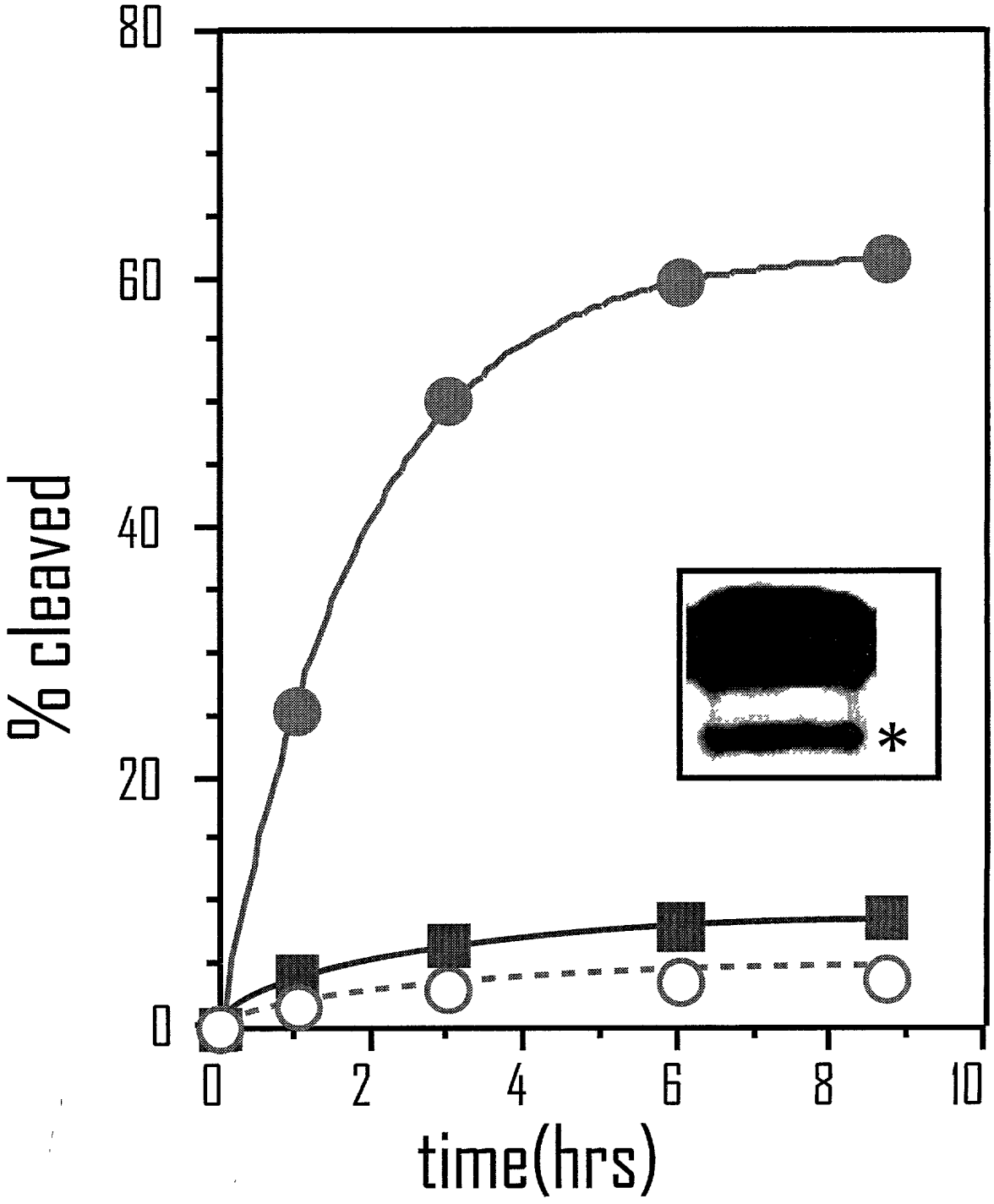


Figure 40A

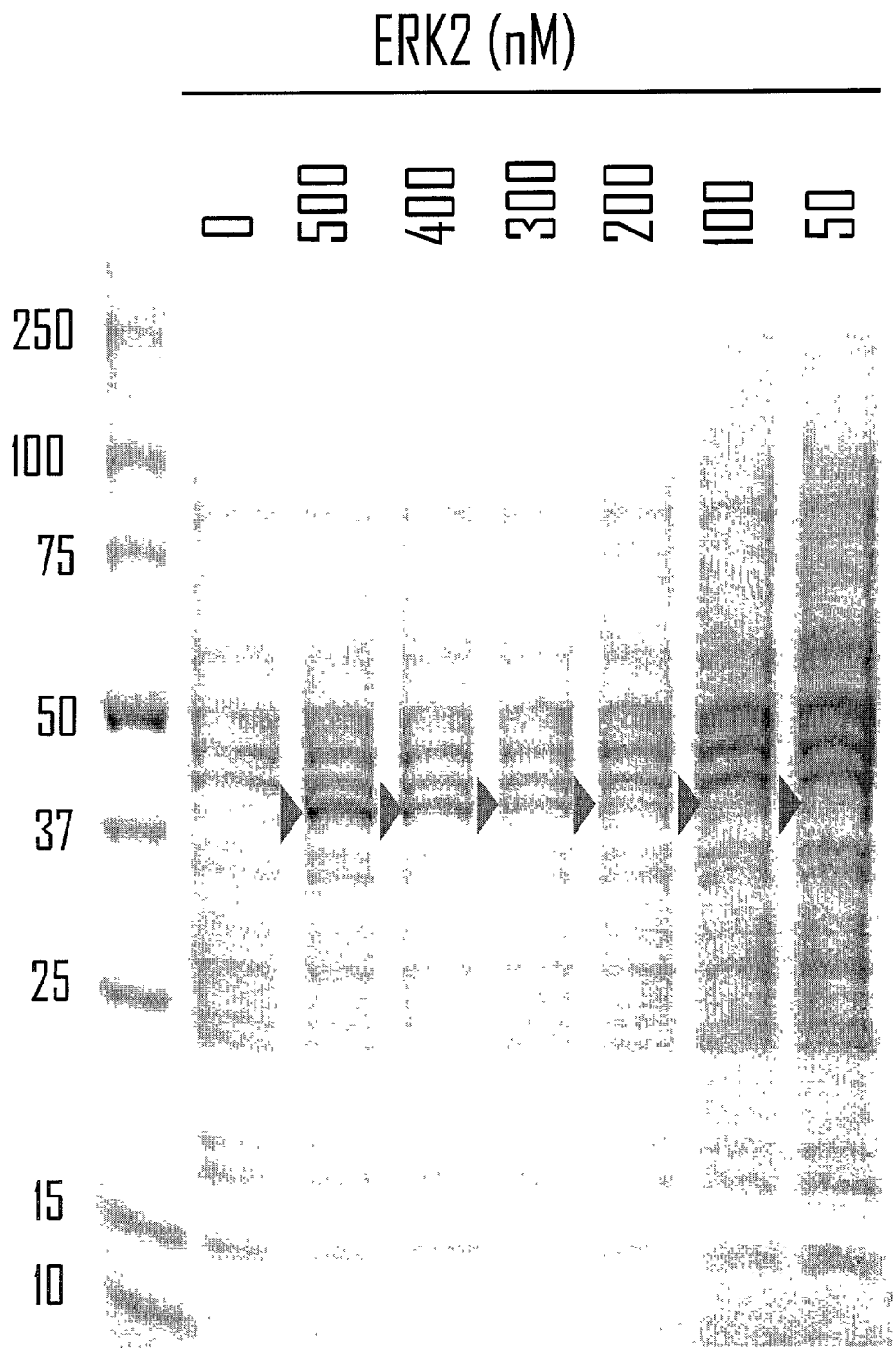


Figure 40B

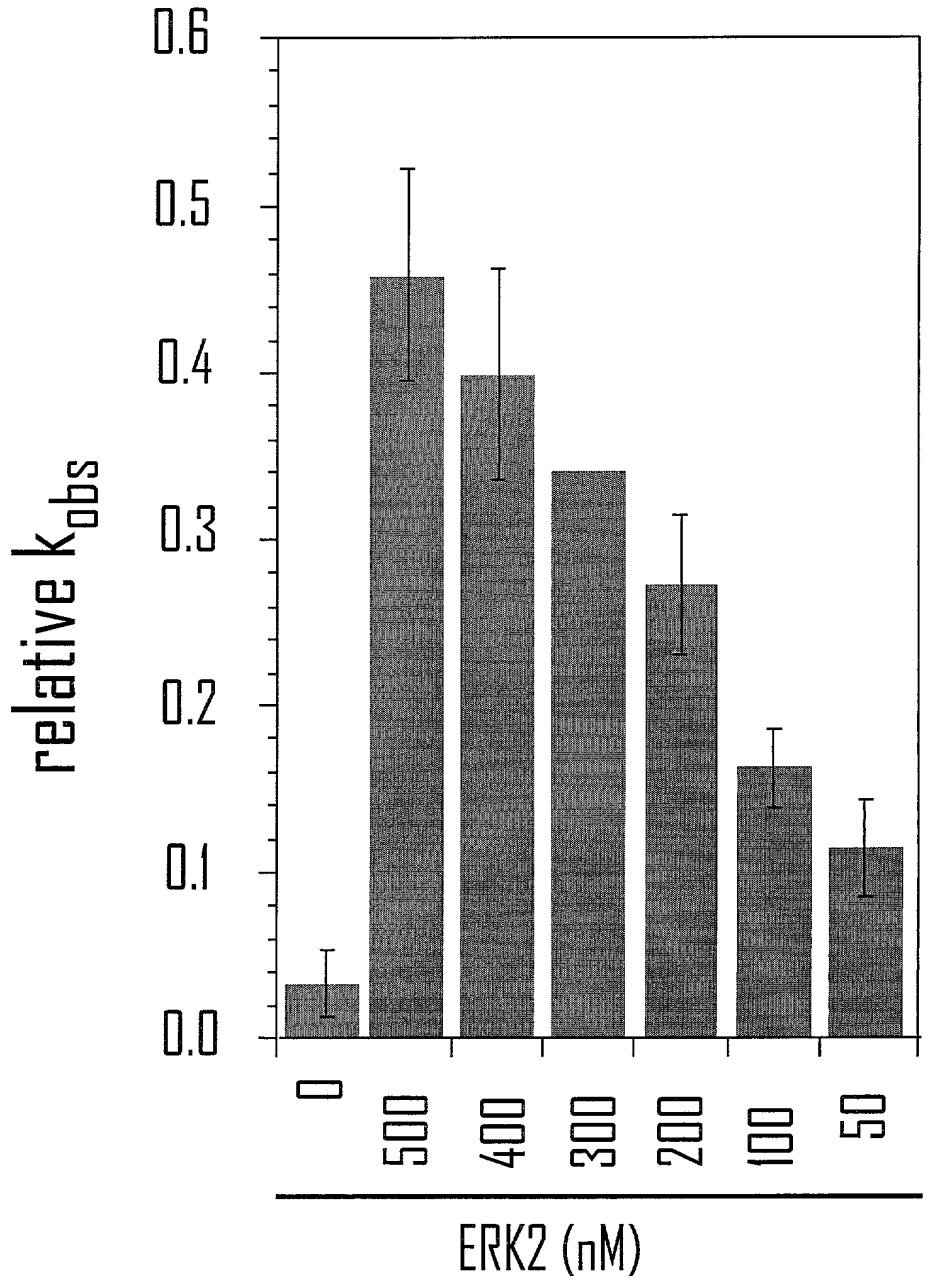

0.6
0.5
0.4
0.3
0.2
0.1
0.0
relative k_{obs}
0
500
400
300
200
100
50
ERK2 (nM)

Figure 41A

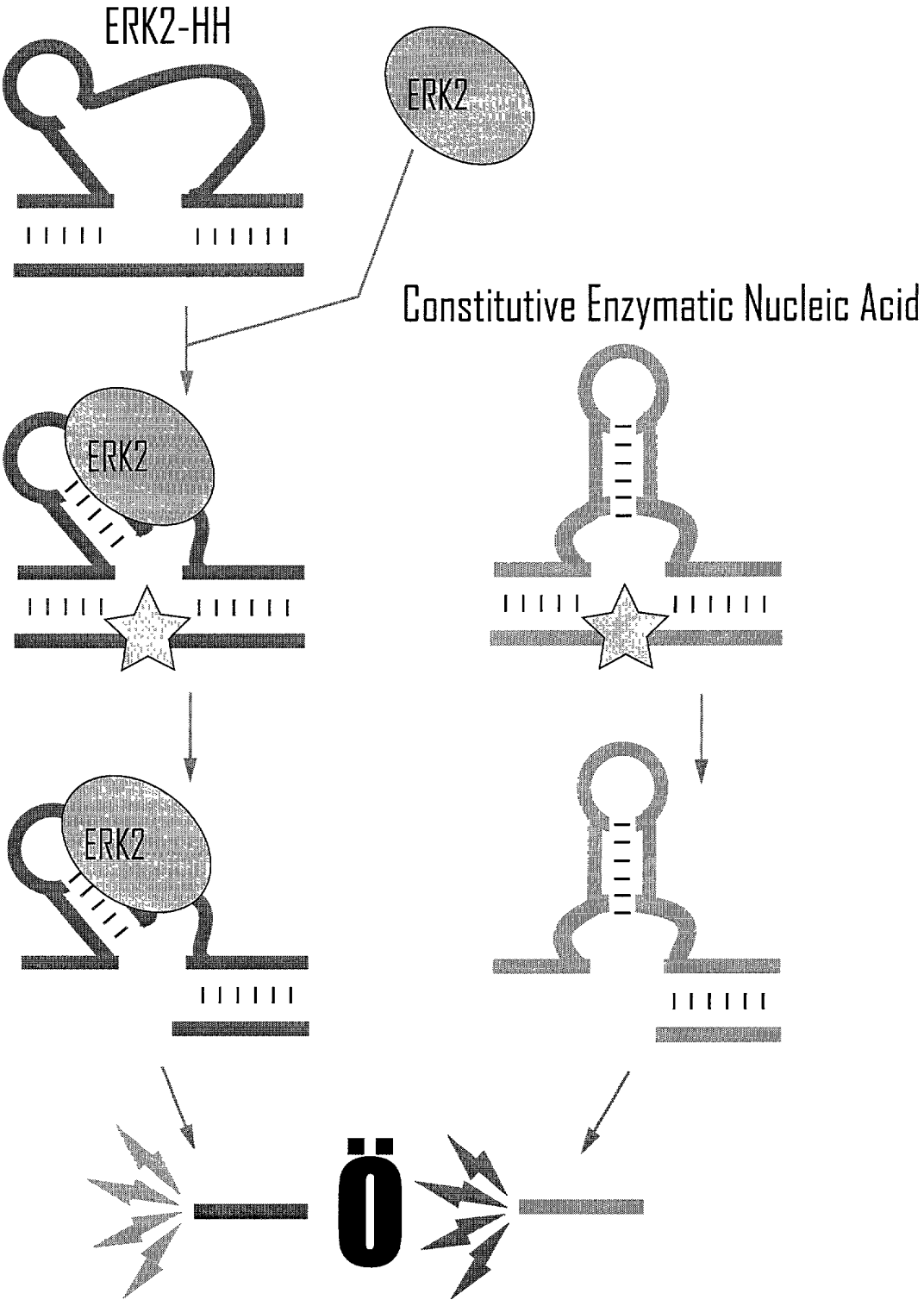

ERK2-HH
ERK2
Constitutive Enzymatic Nucleic Acid
ERK2
ERK2

Figure 41B

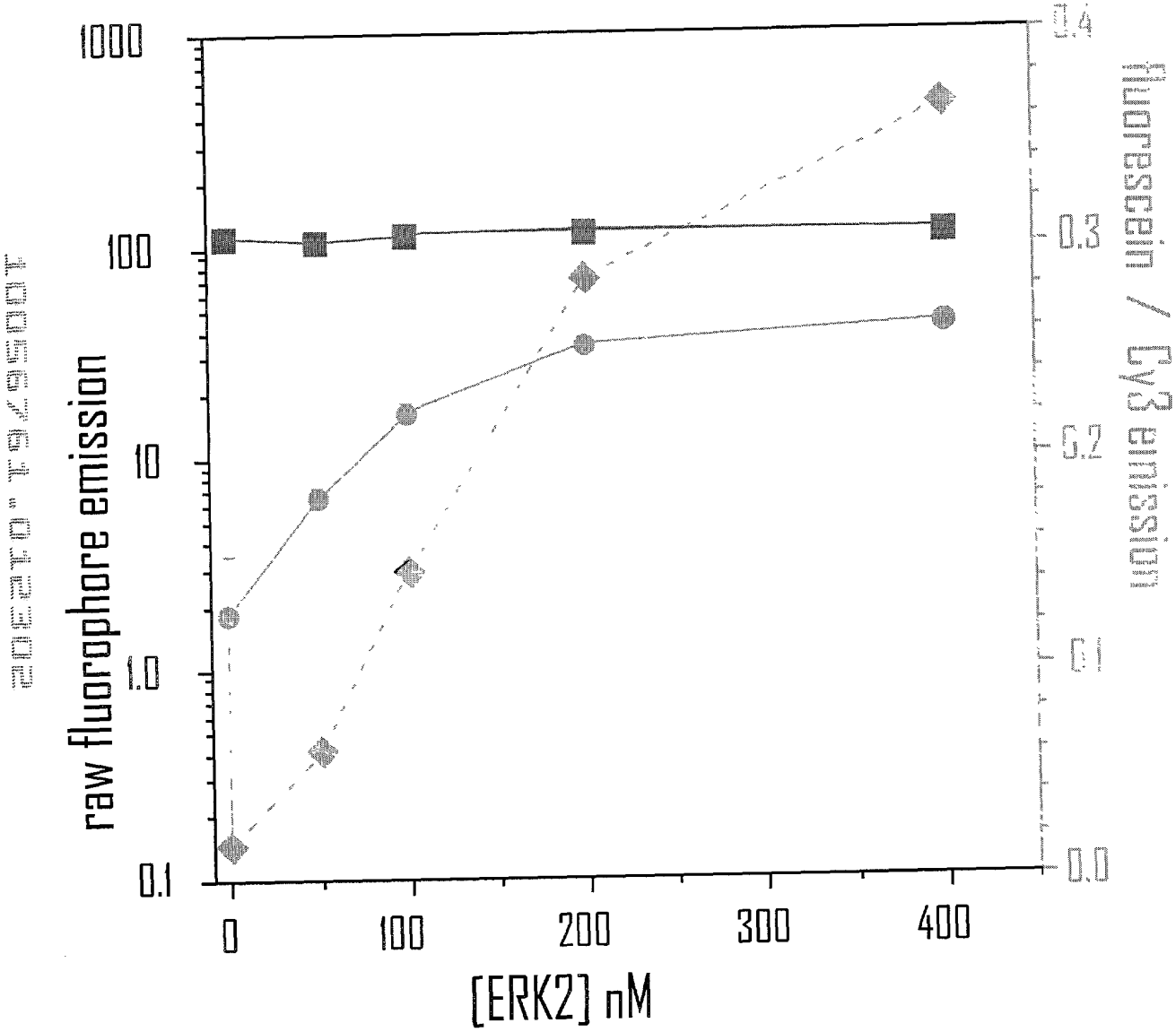
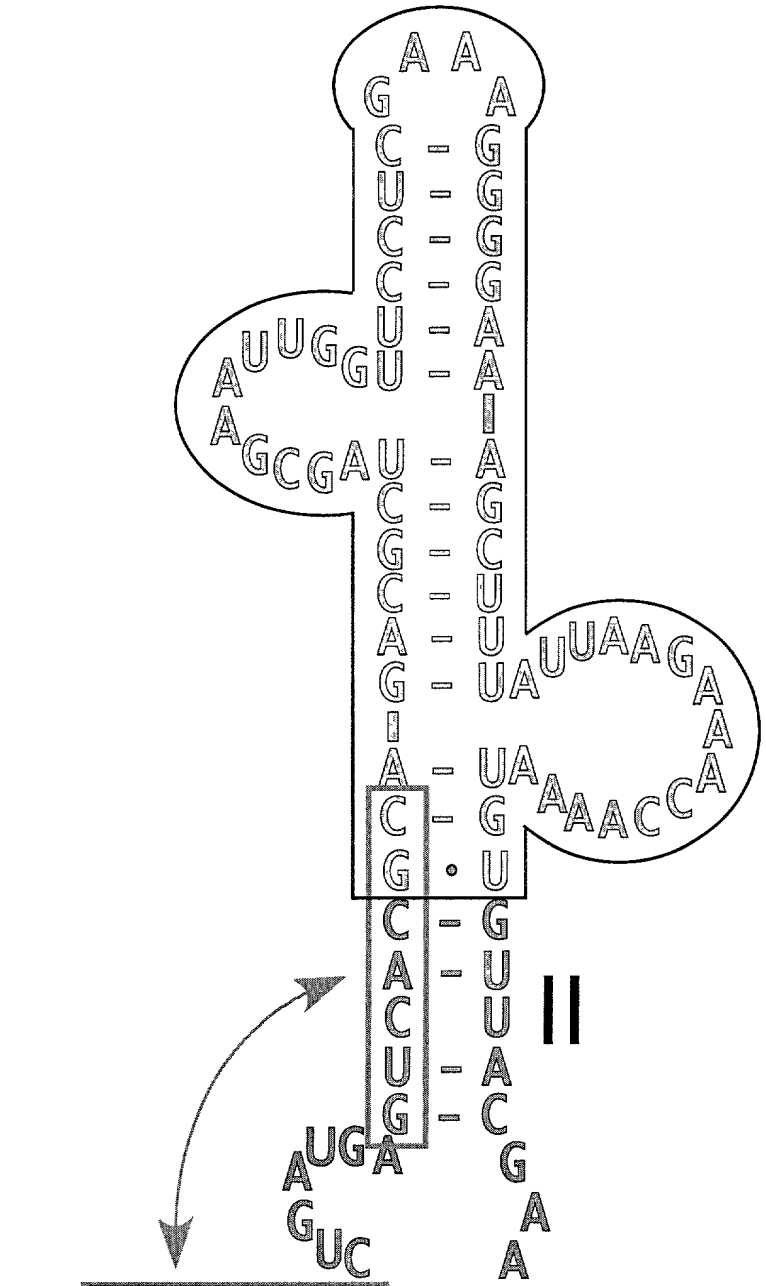

1000
100
10
1.0
0.1
raw fluorophore emission
0.4
0.3
0.2
0.1
0.0
fluorescein / Cy3 emission
0
100
200
300
400
[ERK2] nM

Figure 42A



5'-GGCGUGAC ACGUUCCC (SEQ ID NO: 63)
CGCACUG C UGCAAGG-5' (SEQ ID NO: 62)

Figure 42B